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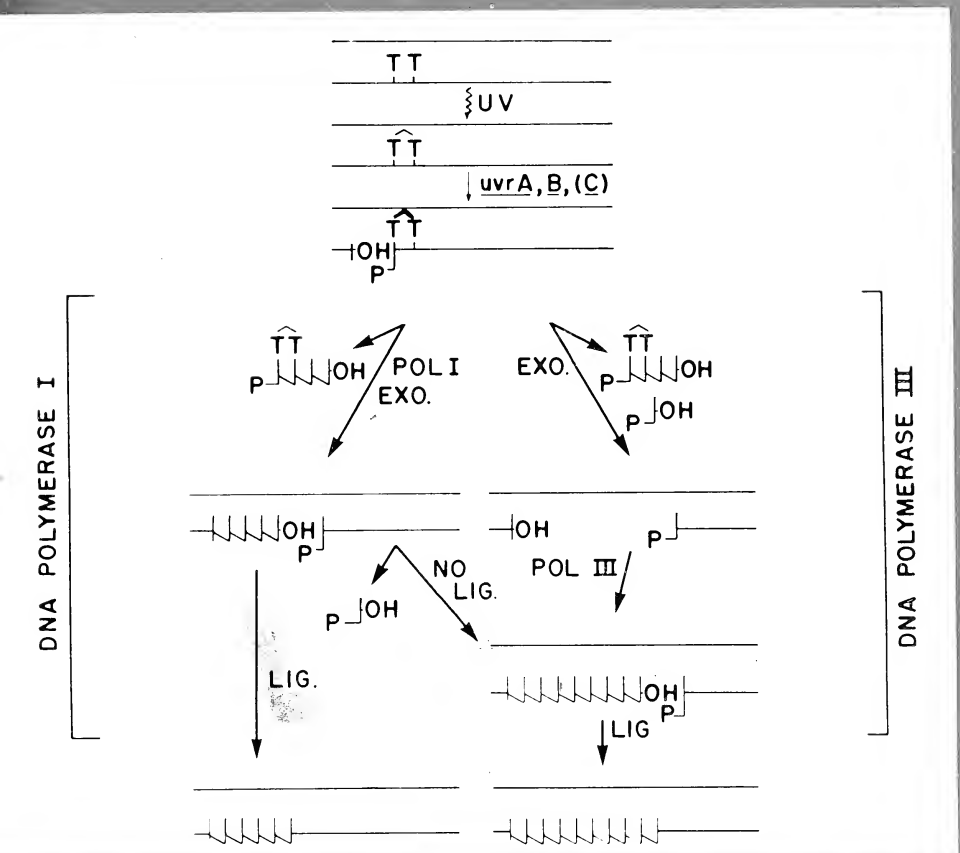

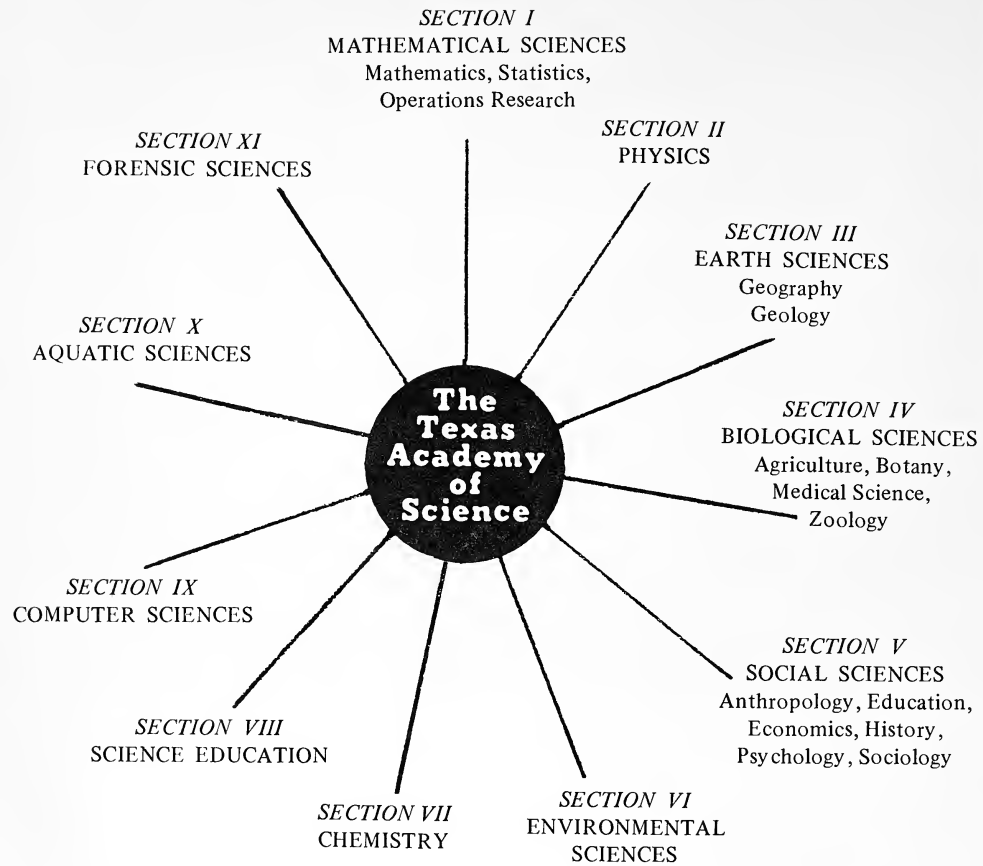


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## INSTRUCTIONS TO AUTHORS

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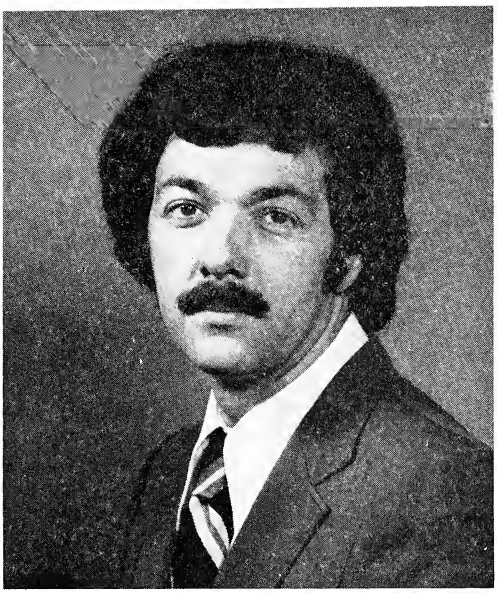

ROBB E. MOSES, M.D.

BIOGRAPHICAL SKETCH

Robb E. Moses received his M.D. degree from the Johns Hopkins University Medical School. While there, he was a Fellow in the laboratory of Dan Nathans. Following an internship in Internal Medicine at Hopkins, he joined Maxine Singer at the National Institutes of Health as a Staff Associate. Subsequently, he was appointed a USPHS Special Fellow with Charles Richardson at Harvard. He has been on the faculty at Baylor College of Medicine in Houston since 1971.

# EXCISION REPAIR FOLLOWING ULTRAVIOLET IRRADIATION IN TOLUENE-TREATED *ESCHERICHIA COLI*

*by* JOHN W. DORSON *and* ROBB E. MOSES

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ABSTRACT

Repair of UV-radiation photoproducts has been examined in bacteria made permeable by exposure to toluene. In toluene-treated cells this repair is due to the excision repair pathway. An active participation in this process by DNA polymerase I is demonstrated by two different experimental approaches. In the first, irradiated cells are allowed to accumulate incisions in the absence of repair synthesis and then repair synthesis is allowed. In wild-type cells this results in a rapid reformation of high molecular weight DNA. The other approach is more direct as the polymerization by DNA polymerase I is directly stimulated. This stimulation is caused by the addition of nicotinamide mononucleotide which blocks polynucleotide ligase activity. This addition causes a large increase in the amount of repair synthesis after UV which is specific for DNA polymerase I. These experiments lead to the conclusion that the repair synthesis catalyzed by DNA polymerase I is an efficient process in which the repair synthesis is easily terminated by polynucleotide ligase and that few nucleotides are inserted into the damage site.

## INTRODUCTION

UV radiation causes the formation of dimers between adjacent pyrimidines on the same DNA strand (Setlow, 1966). There are at least 3 mechanisms for the repair of these dimers (Hanawalt and Setlow, 1975). Photo-reactivation is the process of *in situ* reversal of the dimers. This enzymatic process requires visible irradiation. Postreplication or recombinational repair requires DNA replication and the *rec* gene products. This process repairs gaps in the daughter strands in regions opposite dimers.Excision repair is a cut and patch process wherein the dimers are removed from the DNA and replaced via repair synthesis. It is this final process which we will discuss in this report.

In the simplest terms, excision repair consists of individual steps of incision, excision, repair synthesis and ligation (Grossman, *et al.*, 1970). Incision is the introduction of a break into the phosphodiester backbone of the DNA near the dimer and is under the control of the *uvr* gene products. Excision is the step in which the dimer is actually removed from the DNA. Repair synthesis then fills in the damaged region with correct nucleotides using the opposite strand as template. These nucleotides are joined to the undamaged DNA strand by polynucleo-

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tide ligase to complete the repair process and return both sequence integrity and intactness to the DNA.

Toluene-treated *Escherichia coli* are permeable to small molecules and present an *in vitro* system for a more thorough examination of the repair processes than *in vivo* (Moses and Richardson, 1970). We have used this system to dissect the repair process into its individual steps and then examine these steps. For example, incision is difficult to study *in vivo* because the nicks that are made are rapidly repaired so that at any time during repair there are actually few nicks present in the DNA. In toluene-treated cells by not supplying the deoxyribonucleoside triphosphates (dNTPs) repair synthesis is blocked and incisions cannot be repaired, thus accumulating. In this way, we have been able to study incision as a discrete step. We have shown that incision requires the presence of ATP and proceeds for about 10 to 15 min at 37 C (Dorson, *et al.*, 1975). In this report we examine the contribution of the DNA polymerases to the repair synthesis.

## MATERIALS AND METHODS

Radioactive materials were obtained from Schwarz/Mann. NMN was obtained from Sigma.

Cells were grown in L-broth (10 g tryptone, 5 g yeast extract and 5 g NaCl) supplemented with 10 $\mu$g/ml thymine. To label DNA, the cells were grown in the same media with the addition of 5 $\mu$c/ml tritiated thymidine.

Cells were toluene-treated as previously described (Moses and Richardson, 1970). Cells were irradiated at a dose of 6 J/m$^2$ min following toluene treatment and immediately placed into reaction mixtures.

### *Sucrose Gradient Sedimentation*

Labeled toluene-treated cells were incubated after irradiation in reaction mixtures which contained no radioactive nucleotides. Following incubation the cells were lysed directly on the top of alkaline sucrose gradients. The gradients were centrifuged for 2 hrs at 25,000 rpm at 20 C. Collection was from the bottom directly onto Whatman Number 3 paper strips which were then washed with TCA and cut into scintillation vials and counted. The data is expressed as % of the total counts recovered from the gradient versus the % of the gradient migrated. Molecular weight determinations were by comparison to ØX174 RF1 DNA.

### *Incorporation Determinations*

To measure DNA synthesis unlabeled cells were incubated in reaction mixtures containing deoxyribonucleoside 5′-triphosphates one of which was radioactive. The incubations were stopped by the addition of TCA and acid-insoluble material collected by filtration. The filters were washed with TCA, placed in scintillation vials and counted. Based on specific radioactivity of the precursor, the incorporation was converted to picomoles.

## RESULTS

### *Sedimentation Analysis*

The complete process of excision repair is comprised of many steps or partial reactions. These steps are tightly coordinated. As excision repair requires the introduction of breaks into DNA it seems that these interruptions are removed as rapidly as possible. The result is that in live cells after UV-irradiation the DNA is remarkably intact at any instant. That this is also the case *in vitro* is shown in Figure 1.

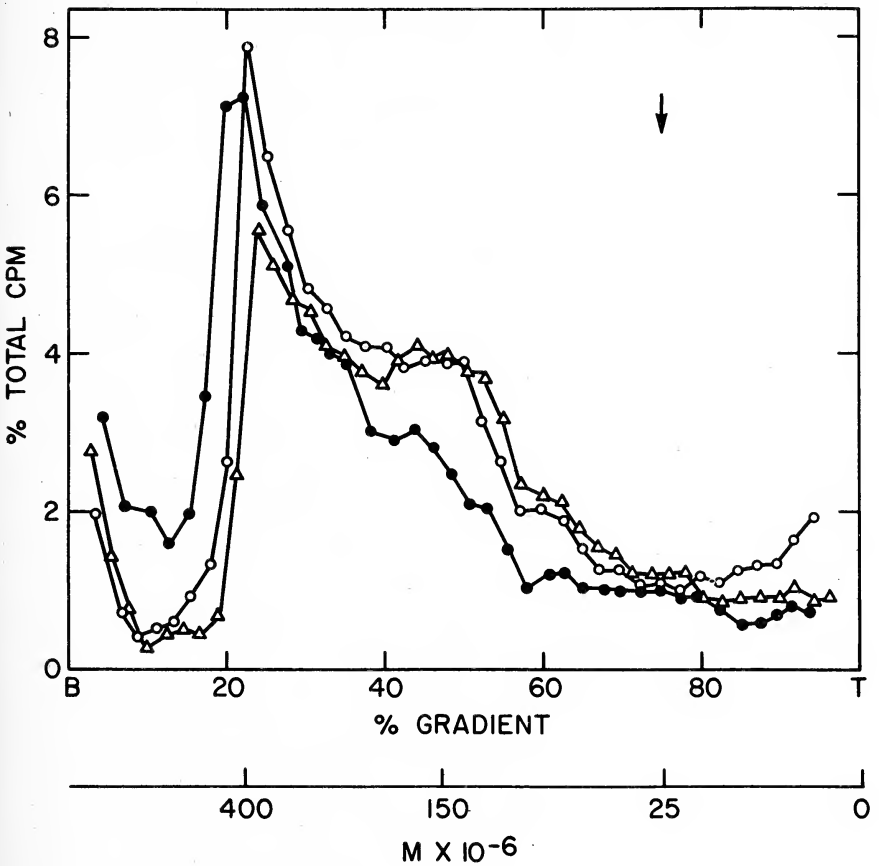


Figure 1. Maintenance of high molecular weight DNA during excision repair when all the requirements are available. Toluene-treated wild-type cells (W3110) were irradiated (30 J/m$^2$) and incubated in reaction mixtures including ATP and dNTPs and stopped at various times. △ = 2 min, ○ = 20 min, ● = unirradiated, 20 min.

After irradiation, toluene-treated wild-type cells incubated in complete reaction mixtures containing the appropriate substrates for DNA synthesis including ATP demonstrate little breakdown in the DNA. This analysis is performed on alkaline sucrose gradients which denature the DNA and is therefore a sensitive indicator of the intactness of the DNA strands.

The rapidity of the complete excision process requires that a block be introduced in order to examine any of the individual steps. By blocking the repair synthesis step any breaks that have been introduced into the DNA may be observed. The arrow (Figure 1) near the top of the gradient indicates the peak position of the counts when repair synthesis is blocked by withholding the deoxyribonucleoside 5′-triphosphates (dNTPs) which are the substrates for the repair synthesis. We previously demonstrated that UV-specific incision requires the presence of ATP (Dorson, *et al.*, 1975). Incisions accumulate in the absence of repair synthesis which is blocked by omitting the dNTPs from the reaction mixtures. This experiment illustrates the efficiency of excision repair in maintaining the intactness of the DNA even though there are breaks being introduced into the DNA.

One way for this process to be so efficient would be for the repair and sealing of the DNA to be very rapid compared to the introduction of the initial incision. Incisions accumulate when irradiated toluene-treated cells are incubated in the presence of ATP but in the absence of dNTPs. The closed circles (Figure 2) are the distribution of the DNA after such an incubation for 10 min. The cell's ability to repair and reseal these breaks is examined by the subsequent addition of dNTPs to allow repair synthesis and the rejoining of the repaired region to the DNA. This rejoining requires the correct configuration in the DNA at the juncture of the break. The results (Figure 2) indicate that as quickly as the first analysis is made (2 min) that the majority of the breaks in the DNA have been repaired and sealed. Even though these breaks required about 10 min to accumulate most are sealed much more quickly indicating the speed of the repair process after the incision has occurred. Further incubation (20 min, open squares) results in a distribution that is very similar to that seen for DNA from unirradiated cells taken through the same protocol (closed squares).

When a similar experiment was performed with *polA*$^-$ cells (DNA polymerase I deficient) there was only a small increase in the molecular weight distribution when the dNTPs were added. Thus, the rapid complete reformation seen in wild type is the result of participation by DNA polymerase I.

*Incorporation Studies*

The activity of the DNA polymerases can be followed by directly measuring the synthesis of DNA during the postirradiation incubation. This incorporation, however, is complicated, due to replicative synthesis. The data in Figure 3 is presented to distinguish the patterns of incorporation due to repair and replication. In the left panel all the incorporation is due to replication as the strain is *uvrA*$^-$ preventing excision repair (Moses and Moody, 1975). Replicative incorporation

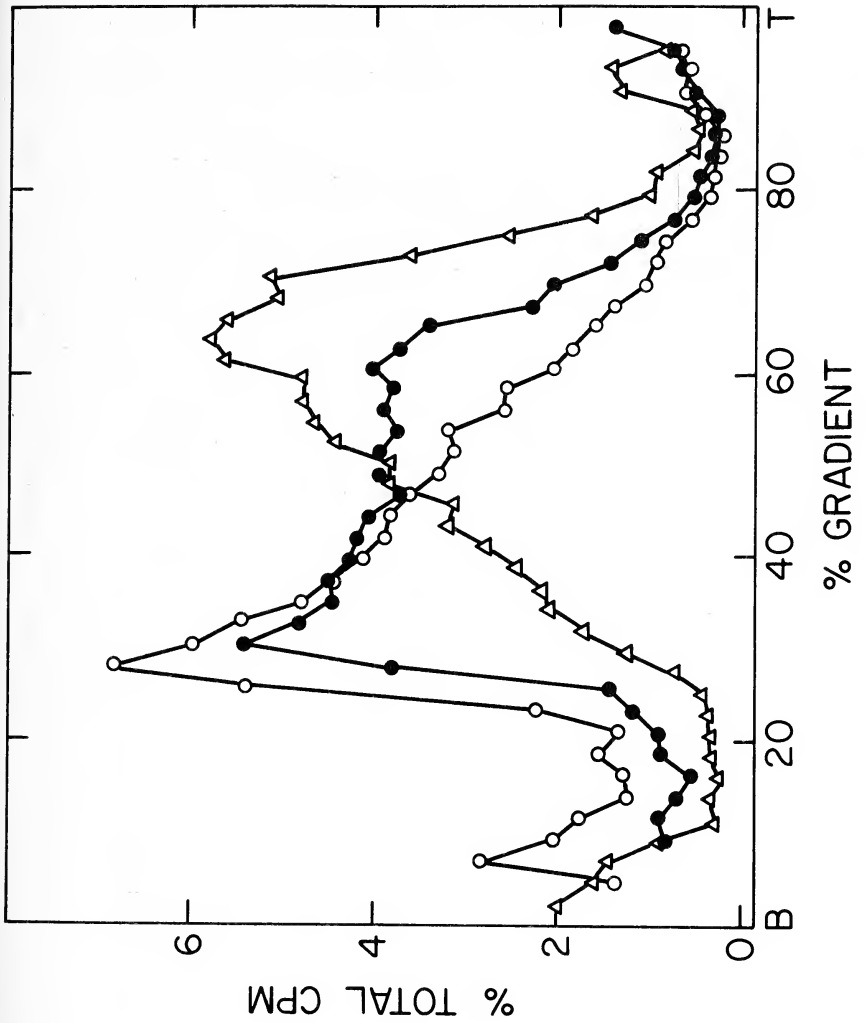


Figure 2. Reformation of high molecular weight DNA subsequent to incision accumulation. Toluene-treated wild-type cells (W3110) were irradiated (30 J/m$^2$) and incubated 5 min in reaction mixtures containing ATP but no dNTPs (△). The dNTPs are then added and incubated for additional time (● = 1 min and ○ = 4 min).

falls as the UV dose is increased indicating the inhibition to replication (Bowersock and Moses, 1973). Repair synthesis is, of course, low until the cells are exposed to UV. This is seen in the center panel when replicative synthesis is blocked in a mutant with a conditional defect in replication, *dnaB*$_{ts}$. As the UV dose increases the repair synthesis also increases until the system appears to saturate and levels off. The result of combining both types of synthesis in the same cell is illustrated

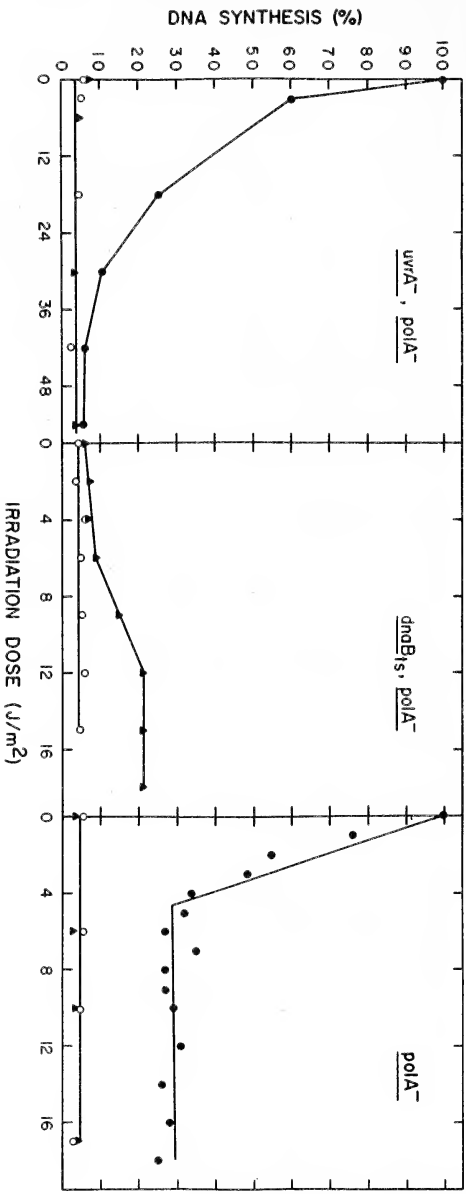


Figure 3. DNA synthesis in toluene-treated cells following irradiation. Toluene-treated cells were exposed to UV light and after various exposures were incubated for 30 min at 37 C in reaction mixtures which contained dNTPs one of which was radioactive (usually 10 cpm/pmole). The strain used in the left panel is *uvrA*⁻, *polA*⁻; the center panel is *dnaB*$_{ts}$, *polA*⁻ at the restrictive temperature; and the right panel is *polA*⁻. ● = complete, left and right; and ▲ = complete, center; ▲ = +NEM, left and right; and ○ = -ATP.

for a *polA*⁻ strain in the right panel. The initial high level of replicative synthesis falls as the UV dose is increased just as before. However, there is now a residual synthesis which is constant at the higher UV exposures. This level of incorporation is seen to be nearly the same as in the center panel in which only repair synthesis occurs. This residual level of synthesis is about thirty percent of the replicative level.

All of the panels in Figure 3 were in *polA*⁻ strains which lack DNA polymerase I. When a comparable experiment is performed in a toluene-treated wild-type strain, the results in Figure 4 are obtained. The level of replicative synthesis is not influenced

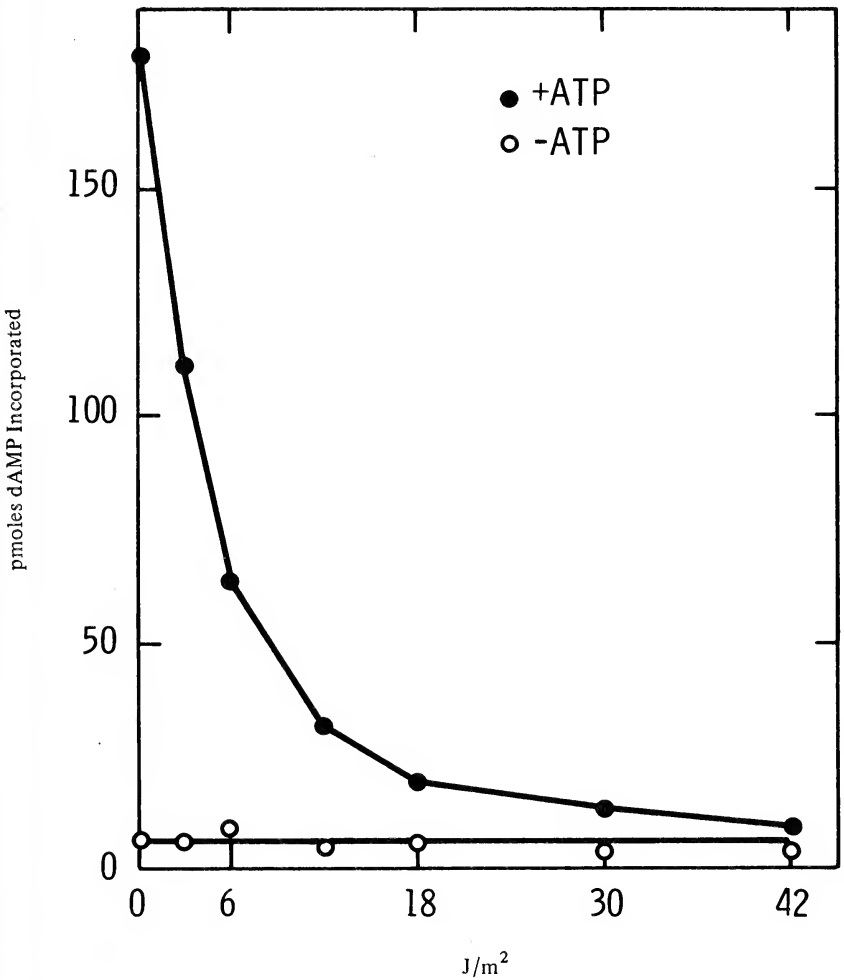


Figure 4. DNA synthesis in toluene-treated wild-type cells (W3110) following irradiation. Experiment performed as described in Figure 3. ● = complete, ○ = -ATP.

by the absence or presence of DNA polymerase I. Once again, the level of incorporation due to replication falls as the UV dose is increased. In a DNA polymerase I-containing strain, however, the level of synthesis that is observed after high UV exposures is very low, in this case near to the background incorporation seen in the absence of ATP.

How can such low levels of repair incorporation be accounted for and is there repair synthesis at all? It was demonstrated in Figure 1 that there is a very efficient repair in wild type. If the number of nucleotides being inserted at each damage site were few enough then the amount of synthesis could be so small as to escape detection. One way for this to occur is by an active participation by polynucleotide ligase. Ligase could seal the nick in the DNA quickly after the dimer had been removed, thus limiting the number of nucleotides inserted. In that event, blocking ligase should lead to a more extensive repair synthesis.

Nicotinamide mononucleotide (NMN) is a product in the ligase activation reaction (Little, *et al.*, 1967). NMN addition reverses the reaction producing an uncharged ligase which is incapable of sealing nicks in DNA. The addition of NMN to toluene-treated cells, blocking ligase activity, results in a large stimulation of repair incorporation (open circles, Figure 5). The addition of NMN is seen to have little effect on the replicative level of synthesis. Increasing the UV exposure first results in a drop in the level of incorporation followed by increasing incorporation at the higher doses. The initial drop is due to the UV being more inhibitory to replication than in stimulating the repair synthesis. At the higher doses the incorporation is almost exclusively repair synthesis.

The NMN-stimulated repair synthesis is ATP dependent as shown by the level of synthesis in its absence (open squares, Figure 5). The ATP independent background level of synthesis is slightly increased by NMN addition but does not vary with changes in the UV exposure. The ATP requirement for repair synthesis can be at least partially explained by the ATP requirement in incision as incision proceeds repair synthesis (Dorson, *et al.*, 1975).

## DISCUSSION

Data presented in this and other publications from our laboratory are summarized in Figure 6. Excision repair is dependent upon the *uvrA, B* gene products which control ATP-dependent incision. The requirements for this initial event are consistent with the observation that other processes which follow incision such as excision and repair synthesis are all ATP and *uvrA, B* dependent (Moses and Moody, 1975; Bowersock and Moses, 1973; Deutsch, *et al.*, 1976). Once incision has occurred, however, the pathway may take 2 possible branches. This branching depends on the presence of DNA polymerase I. Purified DNA polymerase I is capable of binding to incisions and removing dimers by a mechanism of nick translation wherein the associated $5'\rightarrow3'$ exonuclease removes nucleotides as the polymerase inserts undamaged ones in their place (Kelly, *et al.*, 1969). *In vivo* nick translation is efficiently terminated by polynucleotide ligase once the dimer region is passed. This accounts for the very rapid reformation observed following incision accumulation in toluene-treated *polA*$^+$ cells. That there are few nucleotides inserted into each patch site explains why we are unable to observe repair synthesis in wild-type cells. A dose of 30 J/m$^2$ produces about 1500 dimers/genome (Deutsch, *et al.*, 1976). At 8 x $10^8$ cells/reaction this corresponds

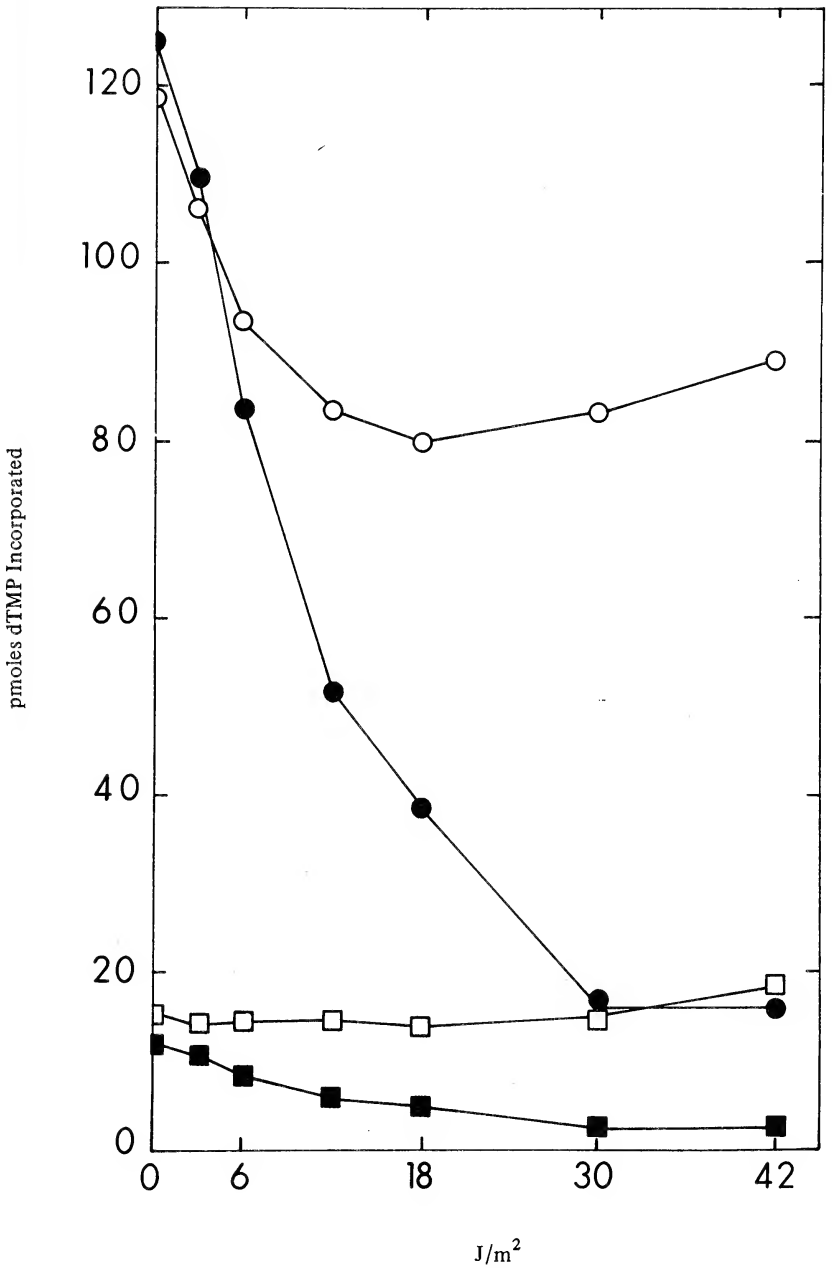


Figure 5. Effect of NMN on DNA synthesis in toluene-treated wild-type cells (W3110) following irradiation. Experiment performed as described in Figure 3. ● = complete; ○ = complete, +NMN; ■ = -ATP; and □ = -ATP, +NMN.

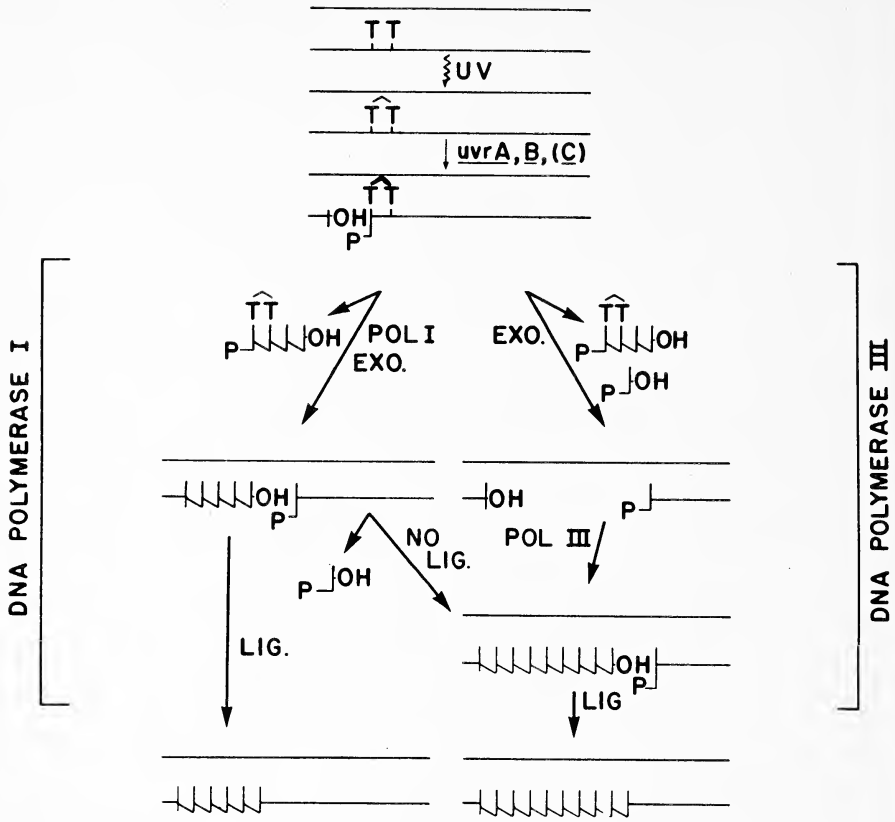


Figure 6. Scheme for excision repair in toluene-treated cells.

to $1.2 \times 10^{12}$ dimers/reaction. If approximately 10 nucleotides are inserted at each damage site *in vitro* as in the case *in vivo* (Cooper and Hanawalt, 1972) then the upper limit for the incorporation in *polA*$^+$ cells would be 20 pmoles. This would occur if all the dimers were repaired. As about 30% of the dimers are actually removed *in vitro* (Deutsch, *et al.*, 1976) this results in an upper limit of 6 to 7 pmoles total incorporation which is too low to distinguish from the ATP-independent background incorporation.

The addition of NMN to toluene-treated cells greatly stimulates the amount of repair synthesis due to DNA polymerase I. NMN does not interact directly with DNA polymerase I nor is it a necessary cofactor or activator. NMN acts by blocking polynucleotide ligase and it is the absence of ligase activity which promotes DNA polymerase I synthesis. The inactivated ligase cannot seal nicks in the DNA during repair and there is nothing to impede DNA polymerase I in extensive nick translation. Conversely, the stimulation by blocking ligase is a direct indication that the DNA contains sealable nicks during the normal repair mediated

by DNA polymerase I. This efficiency in sealing the DNA polymerase I repair is independently shown by the rapid and nearly complete reformation of the DNA in Figure 2.

The 2 branches for DNA polymerase I involvement in excision repair in toluene-treated cells are consistent with *in vivo* situations. In *polA*$^+$ cells the repair patch is short being only about 10 nucleotides whereas the patch in *polA*$^-$ is about 1,000 nucleotides or a long patch (Cooper and Hanawalt, 1972). Therefore, the level of repair synthesis *in vitro* parallels this same pattern, being more extensive in the *polA*$^-$ strains.

The branch in the *polA*$^-$ strains is referred to as the DNA polymerase III pathway (Figure 6). The data supporting this contention is presented elsewhere (Bowersock and Moses, 1973). However, the mechanism for this branch is distinctly different than the nick translation mechanism in *polA*$^+$ cells. There is no stimulation by the addition of NMN to *polA*$^-$ strains (Dorson and Moses, 1975) and the breaks are not as readily sealed. This had led us to suggest that the probable DNA structure during DNA polymerase III repair is one containing a gap with several to a few hundred nucleotides actually removed before the polymerase begins acting. This most likely is the result of an independent exonuclease being responsible for excision and removal of nucleotides past the damage. The physical disconnection of the excising nuclease from the polymerase supposedly would not allow the tight coupling or concerted action. The final step in either branch of the pathway is the rejoining of the DNA break by polynucleotide ligase.

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# MARKOV-RENEWAL PROGRAMMING UNDER INCOMPLETE INFORMATION – I

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## INTRODUCTION

Multistage decision processes have been the subject of active research during the last decade. The scope of sequential decision making has grown with the development of new mathematical models to represent practical situations. This paper deals with the optimization of the output of a system whose behavior is represented by a Markov-renewal process—while the structure of this mathematical model is know, the decision maker is assumed to be operating with incomplete information about the parameters of the mode.

### *Statement of the Problem*

Consider a Markov-renewal process with rewards described by the quadruplet P, F, R, and Z where

| | |
|---|---|
| $P = \{p_{ij}^z\}$ | is the conditional transition probability matrix $(i, j = 1, 2, \ldots, N)$, |
| $F = \{F_{ij}^z(t)\}$ | is the matrix of transition interval distribution functions, |
| $R = \{R_{ij}^z(t \mid \tau(i,j))\}$ | is the matrix of rewards due to the transition from i to j in the clock time $t, 0 \leq t \leq \tau(i,j)$, since the beginning of the transition, |
| $Z = \{1, 2, \ldots, z, . , Z_i\}$ | is the set of alternatives available to the decision maker when the system is in state $i, i = 1, 2, \ldots, N$. |

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A Markov-renewal decision problem is denoted by MRD in the sequel. If an MRD involves incomplete information about P and/or F, they are treated as random variables and are designated by a tilde ($\sim$) placed above those parameters. The lack of information about the parameters of a Markov-renewal process leads to 3 distinct MRD's, namely:

1. MRD: $\{\tilde{P}, F, R, Z\}$
2. MRD: $\{P, \tilde{F}, R, Z\}$
3. MRD: $\{\tilde{P}, \tilde{F}, R, Z\}$

*Optimization Under Incomplete Information About Transition Probabilities*

Consider an MRD: $\{\tilde{P}, F, R, Z\}$. Let the prior distribution of $\tilde{P}$ be $G(P|\mu)$ with the parameter $\mu$ and where G belongs to the family of distributions $\Gamma$ which is closed under consecutive sampling rule. Additionally, assume that the 1st and 2nd moments of the transition interval distributions are finite and nontrivial.

The expected discounted return due to the transition from state i to state j under the $z^{th}$ alternative in i is

$$\rho_{ij}^{z}(\alpha) = \int_0^{\infty} dF_{ij}^{z}(\tau) \cdot \int_0^{\tau} e^{-\alpha x} \cdot d_x R_{ij}^{z}(x|\tau) \tag{1}$$

where a continuous discounting, $e^{-\alpha x}$ with $\alpha > 0$, is used.

The average 1-step discounted return when starting from state i and with the knowledge of $\tilde{P}$ being indexed by $\mu$ is

$$\begin{aligned} \rho_i^z(\alpha, \mu) &= \sum_{j=1}^{N} \rho_{ij}^{z}(\alpha) \cdot \int_{s_{ZN}} \tilde{p}_{ij}^{z}(\mu) \cdot dG(P|\mu) \\ &= \sum_{j=1}^{N} \rho_{ij}^{z}(\alpha) \cdot \bar{p}_{ij}^{z}(\mu) \end{aligned} \tag{2}$$

Here, $\bar{p}_{ij}^{z}(\mu)$ is the expected value of the probability of making a transition from state i to state j under the $z^{th}$ alternative in i.

Let $v_i(\mu)$ be the maximum expected discounted reward over an infinite period when the system starts from the generalized state $(i, \mu)$. Then, each $v_i(\mu)$ must satisfy the following simultaneous functional equations:

$$v_i(\mu) = \max_{z \in Z_i} \{\rho_i^z(\alpha, \mu) + \sum_{j=1}^{N} \bar{p}_{ij}^{z}(\mu) \cdot \int_0^{\infty} dF_{ij}^{z}(t) \cdot e^{-\alpha t} v_j(T_{ij}^{z}(\mu))\} \tag{3}$$

for $i = 1, 2, \ldots, N$; $\mu \in M$; and $\alpha > 0$.

Now, define the functions $v_i(n, \mu)$ recursively as follows:

$$v_i(n, \mu) = \max_{z \in Z_i} \{\rho_i^z(\alpha, \mu) + \sum_{j=1}^{N} \bar{p}_{ij}^{z}(\mu) \cdot \beta_{ij}^{z}(\alpha) \cdot v_j(n-1, T_{ij}^{z}(\mu))\} \tag{4}$$

for $i = 1, 2, \ldots, N$; $\mu \in M$; $n = 1, 2, \ldots$; $\alpha > 0$

where $\{v_i(0,\mu)\} = \{V_i(\mu)\}$ is a set of bounded terminal rewards, and

$$\beta_{ij}^z(\alpha) = \int_0^\infty e^{-\alpha t} \cdot dF_{ij}^z(t) \tag{5}$$

$$= \int_0^\infty e^{-\alpha t} \cdot f_{ij}^z(t)dt \tag{6}$$

if $f_{ij}^z(t)$ exists. It will be shown that $\{v_i(n,\mu)\}$ forms successive approximations to $\{v_i(\mu)\}$. It should be noted that $0 \leq \beta_{ij}^z(\alpha) < 1$ for all $(i, j, z)$ whenever $\alpha > 0$, for, if

$$\beta_{ij}^z(\alpha) = \int_{t_1}^{t_2} e^{-\alpha t} dF_{ij}^z(t), \quad 0 \leq t_1 < t_2 < \infty$$

then, by the mean value theorem, there is a point $t^*$ such that $t_1 < t^* < t_2$, for which

$$\beta_{ij}^z(\alpha) = e^{-\alpha t^*} \int_{t_1}^{t_2} dF_{ij}^z(t) = e^{-\alpha t^*} < 1.$$

*Lemma 1*

Let V and R* be the bounds defined as follows:

$$\left|V_i(\mu)\right| \leq V,$$

$$R^* = \max_{i,\,z} \{ \left|\rho_i^z(\alpha, \mu)\right| \} \quad \text{for } i = 1, 2, \ldots, N;\ z \in Z_i$$

Then, the functions $v_i(n, \mu)$ are bounded:

$$\left|v_i(n, \mu)\right| \leq \beta^n V + \frac{1-\beta^n}{1-\beta} R^* \tag{7}$$

where $$\beta = \max_{i,\,j,\,z} \{\beta_{ij}^z(\alpha)\} \tag{8}$$

Proof: The hypothesis of the lemma is trivially true for n = 0. Assume the lemma for n and let

$$v_i(n+1, \mu) = \rho_i^*(\alpha, \mu) + \sum_{j=1}^{N} \bar{p}_{ij}^*(\mu) \cdot \beta_{ij}^*(\alpha) \cdot v_j(n, T_{ij}^*(\mu))$$

Then,

$$\begin{aligned}
\left|v_i(n+1, \mu)\right| &\leq \left|\rho_i^*(\alpha, \mu)\right| + \beta \sum_{j=1}^{N} \bar{p}_{ij}^*(\mu) \cdot \left|v_j(n, T_{ij}^*(\mu))\right| \\
&\leq R^* + \beta\{\beta^n V + \frac{1-\beta^n}{1-\beta} R^*\} \sum_{j=1}^{N} \bar{p}_{ij}^*(\mu) \\
&= \frac{1}{1-\beta}(R^* - \beta R^* + \beta R^* - \beta^{n+1} R^*) + \beta^{n+1} V \\
&= \beta^{n+1} V + \frac{1-\beta^{n+1}}{1-\beta} R^*.
\end{aligned}$$

That is,

$$|v_i(n+1,\mu)| \le \beta^{n+1}V + \frac{1-\beta^{n+1}}{1-\beta}R^*.$$

Hence, by induction, the lemma is true for all $n \ge 0$.

*Lemma 2*

If V, R*, $\beta$ and the set of functions $\{v_i(n,\mu)\}$ are defined as in earlier equations, then,

$$|v_i(n,\mu) - v_i(m,\mu)| \le (\beta^n + \beta^m)V + \frac{\beta^m - \beta^n}{1-\beta}R^* \tag{9}$$

for all $m<n$, $i = 1, 2, \ldots, N$.

Proof: From Lemma 1 we have that

$$|v_i(n,\mu) - v_i(0,\mu)| \le (1 + \beta^n)V + \frac{1-\beta^n}{1-\beta}R^*.$$

Now, assume the hypothesis of this lemma to be true for m. Then,

$$\begin{aligned} |v_i(n,\mu) - v_i(m+1,\mu)| &\le \beta^n V + \frac{1-\beta^n}{1-\beta}R^* - R^* \\ &\quad + \beta\{\beta^m V - \frac{1-\beta^m}{1-\beta}R^*\} \\ &= (\beta^n + \beta^{m+1})V + \frac{1-\beta^n}{1-\beta}R^* \\ &\quad - R^*\{\frac{1-\beta+\beta-\beta^{m+1}}{1-\beta}\}. \end{aligned}$$

Therefore,

$$|v_i(n,\mu) - v_i(m+1,\mu)| \le (\beta^n + \beta^{m+1})V + \frac{\beta^{m+1} - \beta^n}{1-\beta}R^*.$$

Hence, by induction, the lemma is true for all $m<n$.

*Theorem 1*

The limit of the sequence $\{v_i(n,\mu)\}$, as n tends to infinity, exists.

Proof: From Lemma 2, it can be seen that

$$|v_i(n,\mu) - v_i(n-1,\mu)| \le (\beta^n + \beta^{n-1})V + \frac{\beta^{n-1} - \beta^n}{1-\beta}R^*.$$

Note that

$$\lim_{n\to\infty}\{(\beta^n + \beta^{n-1})V + \frac{\beta^{n-1} - \beta^n}{1-\beta}R^*\} = 0.$$

That is, for any $\varepsilon > 0$, there exists an $n^*$ such that for all $n > n^*$

$$\left|(\beta^n + \beta^{n-1})V + \frac{\beta^{n-1} - \beta^n}{1-\beta}R^*\right| < \varepsilon.$$

This implies that, for all $n > n^*$

$$\left|v_i(n,\mu) - v_i(n-1,\mu)\right| < \varepsilon, \quad i = 1, 2, \ldots, N$$

Satisfying the cauchy criterion for the convergence of the sequence $\{v_i(n,\mu)\}$.

*Theorem 2*

Let

$$v^* = \min_i \{V_i(\mu)\}; \qquad V^* = \max_i \{V_i(\mu)\};$$

$$r^* = \min_{i,z} \{\rho_i^z(\alpha,\mu)\}; \qquad R^* = \max_{i,z} \{\rho_i^z(\alpha,\mu)\};$$

and

$$\beta' = \min_{i,j,z} \{\beta_{ij}^z(\alpha)\}, \qquad \beta'' = \max_{i,j,z} \{\beta_{ij}^z(\alpha)\}.$$

If $r^* + \beta' v^* \geq V^*$, then $\{v_i(n,\mu)\}$ increases monotonically to $\{v_i(\mu)\}$; and if $R^* + \beta'' V^* \leq v^*$, then $\{v_i(n,\mu)\}$ decreases monotonically to $\{v_i(\mu)\}$ as n increases.

Proof: Case 1, $r^* + \beta' v^* \geq V^*$

It is clear that

$$v_i(1,\mu) - v_i(0,\mu) = \max_{z \in Z_i} \{\rho_i^z(\alpha,\mu) + \sum_{j=1}^{N} \bar{p}_{ij}^z(\mu) \cdot \beta_{ij}^z(\alpha) \cdot v_j(0, T_{ij}^z(\mu))\} - V_i(\mu)$$

$$\geq r^* + \beta' v^* - V^* \geq 0 \qquad i = 1, 2, \ldots, N.$$

Now, assume the theorem for n and let

$$v_i(n+1,\mu) = \rho_i^a(\alpha,\mu) + \sum_{j=1}^{N} \bar{p}_{ij}^a(\mu) \cdot \beta_{ij}^a(\alpha) \cdot v_j(n, T_{ij}^a(\mu))$$

and

$$v_i(n,\mu) = \rho_i^b(\alpha,\mu) + \sum_{j=1}^{N} \bar{p}_{ij}^b(\mu) \cdot \beta_{ij}^b(\alpha) \cdot v_j(n-1, T_{ij}^b(\mu)).$$

Then,

$$v_i(n+1,\mu) - v_i(n,\mu) = (\rho_i^a(\alpha,\mu) - \rho_i^b(\alpha,\mu)) + \sum_{j=1}^{N} \bar{p}_{ij}^a(\mu) \cdot \beta_{ij}^a(\alpha) \cdot v_j(n, T_{ij}^a(\mu)) - \sum_{j=1}^{N} \bar{p}_{ij}^b(\mu) \cdot \beta_{ij}^b(\alpha) \cdot v_j(n-1, T_{ij}^b(\mu))$$

$$\geq \sum_{j=1}^{N} \bar{p}_{ij}^b(\mu) \cdot \beta_{ij}^b(\alpha) \cdot \{v_j(n, T_{ij}^b(\mu)) - v_j(n-1, T_{ij}^b(\mu))\}$$

$$\geq 0 \qquad i = 1, 2, \ldots, N.$$

Hence, by induction, the theorem is true for all $n \geq 0$.

Case 2, $R^* + \beta'' V^* \leq v^*$
It is seen that

$$v_i(1,\mu) - v_i(0,\mu) = \max_{z \in Z_i} \{\rho_i^z(\alpha,\mu) + \sum_{j=1}^{N} \bar{p}_{ij}^z(\mu) \cdot \beta_{ij}^z(\alpha) \cdot v_j(0, T_{ij}^z(\mu))\} - V_i(\mu)$$

$$\leq R^* + \beta'' V^* - v^* \leq 0$$

The remainder of the proof follows along similar lines as for Case 1.

Now, letting n approach infinity, it is seen that $v_i(n,\mu)$ converges monotonically to $v_i(\mu)$ for all $i = 1, 2, \ldots, N$, thus proving the theorem.

In the preceeding 2 lemmas and 2 theorems, the existance of an optimal solution to the MRD with incomplete information about the transition probabilities has been established. Theorem 4 also establishes a set of sufficient conditions under which the functions $\{v_i(n,\mu)\}$ converge monotonically to $\{v_i(\mu)\}$.

The recursive equations (4) can be used to compute $\{v_i(n,\mu)\}$ iteratively for $n = 1, 2, \ldots$ when the terminal functions $\{V_i(\mu)\}$ are specified. In using this successive approximation technique, it is necessary to develop a reliable rule for stopping the iterations. The sequence $\{v_i(n,\mu)\}$ has been shown to converge to $\{v_i(\mu)\}$, but only sufficient conditions were developed for its monotonicity. A more acceptable stopping rule is established in Theorem 3 which follows Lemma 3 which is needed first.

*Lemma 3*

Let $r^*$, $R^*$, $\beta'$, and $\beta''$ be defined as before. Then

$$\frac{r^*}{1-\beta'} \leq v_i(\mu) \leq \frac{R^*}{1-\beta''} \qquad (10)$$

$i = 1, 2, \ldots, N.$

Proof: The mean reward per transition must satisfy

$$r^* \leq \rho_i^z(\alpha, \mu) \leq R^*$$

$$i = 1, 2, \ldots, N \qquad z \in Z_i$$

If the system is run over an infinite period, the bounds on $v_i(\mu)$ are obtained by summing the bounds on $\rho_i^z(\alpha, \mu)$ with the appropriate discounting. Thus,

$$\sum_{n=0}^{\infty} r^*(\beta')^n \leq v_i(\mu) \leq \sum_{n=0}^{\infty} R^*(\beta'')^n$$

or

$$\frac{r^*}{1-\beta'} \leq v_i(\mu) \leq \frac{R^*}{1-\beta''} \qquad i = 1, 2, \ldots, N.$$

*Theorem 3*

Let the terminal functions $\{V_i(\mu)\}$ be constants and let $e_i(n, \mu)$ be the error of the $n^{th}$ approximant. That is, let

$$e_i(n, \mu) = v_i(\mu) - v_i(n, \mu) \qquad i = 1, 2, \ldots, N. \tag{11}$$

Then

$$|e_i(n, \mu)| \leq (\beta'')^n \{\max(\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'})\}. \tag{12}$$

Proof: When $n = 0$,

$$e_i(0, \mu) = v_i(\mu) - V_i.$$

If $v_i(\mu) \geq V_i$, then

$$|e_i(0, \mu)| \leq \frac{R^*}{1-\beta''} - v^*.$$

On the other hand, if $v_i(\mu) < V_i$, then

$$|e_i(0, \mu)| = V_i - v_i(\mu) \leq V^* - \frac{r^*}{1-\beta'}$$

Hence, $|e_i(0, \mu)| \leq \max\{\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'}\}$.

Having established the theorem for $n = 0$, assume that it holds $n$ and let

$$v_i(\mu) = \rho_i^a(\alpha, \mu) + \sum_{j=1}^{N} \bar{p}_{ij}^a(\mu) \cdot \beta_{ij}^a(\alpha) \cdot v_j(T_{ij}^a(\mu))$$

and

$$v_i(n=1,\mu) = \rho_i^b(\alpha,\mu) = \sum_{j=1}^{N} \bar{p}_{ij}^b(\mu) \cdot \beta_{ij}^b(\alpha) \cdot v_j(n, T_{ij}^b(\mu))$$

Thus,

$$\sum_{j=1}^{N} \bar{p}_{ij}^b(\mu) \cdot \beta_{ij}^b(\alpha)[v_j(T_{ij}^b(\mu)) - v_j(n, T_{ij}^b(\mu))]$$

$$\leq e_i(n+1,\mu)$$

$$\leq \sum_{j=1}^{N} \bar{p}_{ij}^a(\mu) \cdot \beta_{ij}^a(\alpha)[v_j(T_{ij}^a(\mu) - v_j(n, T_{ij}^a(\mu))]$$

Let $\left| \sum_{j=1}^{N} \bar{p}_{ij}^*(\mu) \cdot \beta_{ij}^*(\alpha)[v_j(T_{ij}^*(\mu)) - v_j(n, T_{ij}^*(\mu))] \right|$ be the larger of the absolute values of the 2 bounds. Then,

$$\left|e_i(n+1,\mu)\right| \leq \sum_{j=1}^{N} p_{ij}^*(\mu) \cdot \beta_{ij}^*(\alpha) \cdot \left|v_j(T_{ij}^*(\mu) - v_j(n, T_{ij}^*(\mu))\right|$$

$$\leq \beta'' [(\beta'')^n \{\max(\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'})\}]$$

That is,

$$\left|e_i(n+1,\mu)\right| \leq (\beta'')^{n+1} \{\max(\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'})\}.$$

Hence, by induction, the theorem is true for all $n \geq 0$.

Note that the sequence

$$(\beta'')^n \{\max(\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'})\}$$

converges monotonically to zero as n approaches infinity even though $\{|e_i(n,\mu)|\}$ need not be a monotonic sequence. Thus, if $\varepsilon > 0$ is an error acceptable to the decision maker, it is possible to compute the smallest n which satisfies

$$(\beta'')^n \{\max(\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'})\} < \varepsilon. \qquad (13)$$

Then so computed gives the maximum number of iterations that need to be done on $\{v_i(n,\mu)\}$ to guarantee an $\varepsilon$-optimal estimate of $\{v_i(\mu)\}$. This can therefore be used effectively as a stopping rule.

In dealing with an MRD: $\{\tilde{P}, F, R, Z\}$, one way of estimating this unknown quantity is on the basis of frequency counts. Consider an MRD from which a sample $X_n = \{x_0, x_1, x_2, \ldots, x_n\}$ of n transitions have been observed under consecutive sampling rule. Here, $x_0$ is the initial state known before sampling. Let

$\nu_{ij}^z$ be the number of transition in $X_n$ from i to j under the $z^{th}$ alternative in i. Let $\nu = \{\nu_{ij}^z\}$ be the Z x N matrix of transition counts. Then, the likelihood of observing $X_n$ given that $\tilde{P} = P$ is

$$\prod_{\substack{i=1\\j=1}}^{N} \prod_{z=1}^{Z_i} (p_{ij}^z)^{\nu_{ij}^z}. \tag{14}$$

It is seen that $\nu$ is a sufficient statistic for this data generating process. The natural conjugate distribution is matrix beta distribution. That is, $\tilde{P}$ has the joint density function with parameter $\mu$,

$$f(P|\mu) = k(\mu) \prod_{\substack{i=1\\j=1}}^{N} \prod_{z=1}^{Z_i} (p_{ij}^z)^{m_{ij}-1} \tag{15}$$

where

$$k(m) = \prod_{i=1}^{N} \prod_{z=1}^{Z_i} \frac{\Gamma(\mu_i^z)}{\prod_{j=1}^{N} \Gamma(\mu_{ij}^z)} \tag{16}$$

and $\mu = \{\mu_{ij}^z\}$ is a Z x N matrix such that

$$\mu_{ij}^z = \nu_{ij}^z + 1 \tag{17}$$

$$\mu_i^z = \sum_{j=1}^{N} \mu_{ij}^z \tag{18}$$

*Theorem 4*

Let $\tilde{P}$ have matrix beta distribution with parameter $\mu'$. Suppose that a sample with transition counts $\nu$ is observed under consecutive sampling rule. Then the posterior distribution of $\tilde{P}$ is also matrix beta with parameter

$$\mu'' = \mu' + \nu \tag{19}$$

Proof: By Bayes' theorem the posterior density function, $f(P|\mu', \nu)$, is proportional to the product of the kernel of the likelihood function and the kernel of the prior density function. Therefore,

$$f(P|\mu', \nu) \propto \prod_{\substack{i=1\\j=1}}^{N} \prod_{z=1}^{Z_i} (p_{ij}^z)^{\mu_{ij}^z + \mu_{ij}^z - 1} \tag{20}$$

The right-hand side of (20) is the kernel of a matrix beta density function with parameter $\mu' + \nu$. Hence the theorem.

The fact that the family of matrix beta distributions is closed under consecutive sampling rule follows directly from this theorem.

If $\tilde{P}$ follows a matrix beta distribution with parameter $\mu = \{\mu_{ij}^z\}$, then

$$\bar{p}_{ij}^z = \frac{\mu_{ij}^z}{\mu_i} . \tag{21}$$

*Example of an MRD:* $\{\tilde{P}, F, R, Z\}$

Consider an MRD with 2 states and 2 alternatives in each state. Let the transition intervals be random variables with the following density functions:

$$f_{ij}^z(t) = \theta_{ij}^z e^{-\theta_{ij}^z t}$$

with

$$\theta = \{\theta_{ij}^z\} = \begin{bmatrix} 5 & 3 \\ 4 & 2 \\ 6 & 4 \\ 10 & 5 \end{bmatrix}$$

Assume that the transition probability matrix P is not known completely but is known to follow matrix beta distribution with parameter $\mu$,

$$\mu = \begin{bmatrix} 3 & 3 \\ 4 & 1 \\ 2 & 3 \\ 8 & 2 \end{bmatrix}$$

When the system is in state i and is being operated under the $z^{th}$ alternative, let the reward accumulated by $r_i^z$ units per unit of time,

$$R = \{r_i^z\} = \begin{bmatrix} 9 \\ 6 \\ -3 \\ -6 \end{bmatrix}$$

The problem is to maximize the total expected discounted reward when the system is operated for an indefinitely long time. Continuous discounting with $\alpha = 40$ is to be used. Now,

$$\rho_{ij}^z(\alpha) = \int_0^\infty dF_{ij}^z(t) \cdot \int_0^t e^{-\alpha x} \cdot d_x R_{ij}^z(x|t)$$

$$= \frac{r_i^z}{\alpha + \theta_{ij}^z}$$

and,

$$\beta_{ij}^{z}(\alpha) = \int_0^{\infty} e^{-\alpha t} dF_{ij}^{z}(t)$$

$$= \frac{\theta_{ij}^{z}}{\alpha + \theta_{ij}^{z}}$$

$$\bar{P}(\mu) = \{\bar{p}_{ij}^{z}(\mu)\} = \begin{bmatrix} 0.5 & 0.5 \\ 0.8 & 0.2 \\ 0.4 & 0.6 \\ 0.8 & 0.2 \end{bmatrix}$$

$$\{\rho_{ij}^{z}(\alpha)\} = \begin{bmatrix} 0.20 & 0.21 \\ 0.14 & 0.14 \\ -0.07 & -0.07 \\ -0.12 & -0.13 \end{bmatrix}$$

$$\{\beta_{ij}^{z}(\alpha)\} = \begin{bmatrix} 0.11 & 0.07 \\ 0.09 & 0.05 \\ 0.13 & 0.09 \\ 0.20 & 0.11 \end{bmatrix}$$

$$\rho_i^{z}(\alpha, \mu) = \sum_{j=1}^{N} \bar{p}_{ij}^{z}(\mu) \rho_{ij}^{z}(\alpha)$$

$$\{\rho_i^{z}(\alpha, \mu)\} = \begin{bmatrix} 0.205 \\ 0.140 \\ -0.070 \\ -0.120 \end{bmatrix}$$

Assume

$$v(0, \mu) = \begin{bmatrix} v_1(0, \mu) \\ v_2(0, \mu) \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$v_i(1, \mu) = \max_{z \in Z_i} \{\rho_i^{z}(\alpha, \mu) + \sum_{j=1}^{N} \bar{p}_{ij}^{z}(\mu) \cdot \beta_{ij}^{z}(\alpha) \cdot v_j(0, T_{ij}^{z}(\mu))\}$$

$$= \max_{z \in Z_i} \{\rho_i^{z}(\alpha, \mu)\}$$

Hence,

$$v(1, \mu) = \begin{bmatrix} 0.205 \\ -0.070 \end{bmatrix}$$

The policy corresponding to this is $\zeta(1,\mu) = \begin{bmatrix} 1 \\ 1 \end{bmatrix}$. Now,

$$v_i(2,\mu) = \max_{z \in Z_i} \{\rho_i^z(\alpha,\mu) + \sum_{j=1}^{N} \bar{p}_{ij}^z(\mu) \cdot \beta_{ij}^z(\alpha) \cdot v_j(1, T_{ij}^z(\mu))\}$$

$i, j = 1, 2;\ z = 1, 2.$

Hence,

$$T_{11}^1(\mu) = \begin{bmatrix} 4 & 3 \\ 4 & 1 \\ 2 & 3 \\ 8 & 2 \end{bmatrix}$$

From this, $\{\rho_i^z(\alpha, T_{11}^1(\mu))\}$ is computed to be

$$\{\rho_i^z(\alpha, T_{11}^1(\mu))\} = \begin{bmatrix} 0.20 \\ 0.14 \\ -0.07 \\ -0.12 \end{bmatrix}$$

which yields

$$v(1, T_{11}^1(\mu)) = \begin{bmatrix} 0.20 \\ -0.07 \end{bmatrix}$$

The following can be computed using a similar procedure:

$$v(1, T_{11}^2(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix};$$

$$v(1, T_{12}^1(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix}; \qquad v(1, T_{12}^2(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix}$$

$$v(1, T_{21}^1(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix}; \qquad v(1, T_{21}^2(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix}$$

$$v(1, T_{22}^1(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix}; \qquad v(1, T_{22}^2(\mu)) = \begin{bmatrix} 0.21 \\ -0.07 \end{bmatrix}.$$

This can be used in computing $v(2,\mu)$ as follows:

$$v_1(2,\mu) = \max \begin{cases} \rho_1^1(\alpha,\mu) + \bar{p}_{11}^1(\mu) \cdot \beta_{11}^1(\alpha) \cdot v_1(1, T_{11}^1(\mu)) \\ \qquad + \bar{p}_{12}^1(\mu) \cdot \beta_{12}^1(\alpha) \cdot v_2(1, T_{12}^1(\mu)) \\ \rho_1^2(\alpha,\mu) + \bar{p}_{11}^2(\mu) \cdot \beta_{11}^2(\alpha) \cdot v_1(1, T_{11}^2(\mu)) \\ \qquad + \bar{p}_{12}^2(\mu) \cdot \beta_{12}^2(\alpha) \cdot v_2(1, T_{12}^2(\mu)) \end{cases}$$

$$= \max \begin{bmatrix} 0.214 \\ 0.154 \end{bmatrix} = 0.214 .$$

Similarly,

$$v_2(2, \mu) = \max \begin{bmatrix} -0.063 \\ -0.088 \end{bmatrix} = -0.063.$$

Thus,

$$v(2, \mu) = \begin{bmatrix} 0.214 \\ -0.063 \end{bmatrix} .$$

The policy corresponding to the 2nd approximation is

$$\zeta(2, \mu) = \begin{bmatrix} 1 \\ 1 \end{bmatrix} .$$

By following a similar procedure, the 3rd approximants are computed. It should be noted that to calculate $v_i(3, \mu)$, it is necessary to compute $v_i(2, T^z_{ij}(\mu))$ which further involves computing $v_i(1, T^z_{k1}(T^z_{ij}(\mu)))$. It can be shown that

$$v(3, \mu) = \begin{bmatrix} 0.214 \\ -0.063 \end{bmatrix}$$

and

$$\zeta(3, \mu) = \begin{bmatrix} 1 \\ 1 \end{bmatrix} .$$

RESULTS

The results are summarized in Table 1. The error bound shown in the last column is computed using the inequality (13). Note that.

$$v^* = V^* = 0; \; R^* = 0.205, \; r^* = -0.120,$$

$$\beta' = 0.05, \; \beta'' = 0.20$$

$$\frac{R^*}{1-\beta''} - v^* = 0.256; \; V^* - \frac{r^*}{1-\beta'} = 0.126 .$$

Therefore, the upper bound on the error of the $n^{th}$ approximant is

$$(\beta'')^n \left\{ \frac{R^*}{1-\beta''} - v^* \right\} = (0.2)^n (0.256) .$$

Thus, $v(\mu)$ can be estimated with a 3rd decimal accuracy by performing 3 iterations.

TABLE 1

Summary of Computations for the Example MRD: $\{\tilde{P}, F, R, Z\}$.

| n | $v(n, \mu)$ | $\zeta(n, \mu)$ | Error Bound |
|---|---|---|---|
| 0 | 0<br>0 | –<br>– | 0.256 |
| 1 | 0.205<br>-0.070 | 1<br>1 | 0.051 |
| 2 | 0.214<br>-0.063 | 1<br>1 | 0.010 |
| 3 | 0.214<br>-0.063 | 1<br>1 | 0.002 |

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# MARKOV-RENEWAL PROGRAMMING UNDER INCOMPLETE INFORMATION – II

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## INTRODUCTION

The Markov-Renewal decision problem was introduced in the preceeding paper, and the case of MRD: $\{\tilde{P}, F, R, Z\}$ was discussed. The other 2 problems, namely MRD: $\{P, \tilde{F}, R, Z\}$ and $\{\tilde{P}, \tilde{F}, R, Z\}$ are presented here. The solution procedure is illustrated by numerical examples.

*Optimization Under Incomplete Information About Transition Interval Distributions*

Consider an MRD: $\{P, \tilde{F}, R, Z\}$. Let the transition interval distributions, $F = \{F_{ij}^z(t/\psi)\}$ indexed on $\psi$, belong to a family $\Phi$ which is closed under consecutive sampling rule.

The expected discounted reward that is accumulated when the system makes a transition from i to j under the $z^{th}$ alternative in i is

$$\rho_{ij}^z(\alpha, \psi) = \int_0^\infty dF_{ij}^z(t/\psi) \cdot \int_0^t \bar{e}^{\alpha x} \cdot d_x R_{ij}^z(x/t). \tag{1}$$

Again, continuous discounting, $\bar{e}^{\alpha x}$ with $\alpha > 0$ is used.

The expected discounted reward when starting from state i and using $z^{th}$ alternative is,

$$\rho_i^z(\alpha, \psi) = \sum_{j=1}^{N} p_{ij}^z \cdot \rho_{ij}^z(\alpha, \psi). \tag{2}$$

When the system is operated for an indefinitely long time, let $v_i(\psi)$ be the maximum total expected discounted reward accumulated when starting from State $(i, \psi)$. Then $v_i(\psi)$ must satisfy the following set of simultaneous functional equations:

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$$v_i(\psi) = \max_{z \in Z_i} \{\rho_i^z(\alpha, \psi) + \sum_{j=1}^{N} p_{ij}^z \cdot \int_0^{\infty} dF_{ij}^z(t/\psi) \cdot \bar{e}^{\alpha t} \cdot v_j(T_t^{iz}(\psi)\} \qquad (3)$$

for $i = 1, 2, \ldots, N; \quad \alpha > 0.$

Here, $T_t^{iz}$ is the mapping of $\psi$ into $\psi$ due to the transition from i under the policy z in an interval of time t.

A sequence $\{v_i(n, \psi)\}$ can be defined recursively as follows:

$$v_i(n, \psi) = \max_{z \in Z_i} \{\rho_i^z(\alpha, \psi) + \sum_{j=1}^{N} p_{ij}^z \int_0^{\infty} dF_{ij}^z(t/\psi) \cdot \bar{e}^{\alpha t} \cdot v_j(n-1, T_t^{iz}(\Psi)\} \qquad (4)$$

$i = 1, 2, \ldots, N.$

Let the range of the transition interval be $0 \leq t_1 < t_2 < \infty$. Then, by mean value theorem, there is a value s such that $t_1 < s < t_2$, and

$$\int_{t_1}^{t_2} dF_{ij}^z(t/\psi) \cdot \bar{e}^{\alpha t} \cdot v_j(n, T_t^{iz}(\psi)) = \bar{e}^{\alpha s} \cdot v_j(n, T_s^{iz}(\psi)) \int_{t_1}^{t_2} dF_{ij}^z(t/\psi).$$

That is,

$$\int_{t_1}^{t_2} dF_{ij}^z(t/\psi) \cdot \bar{e}^{\alpha t} \cdot v_j(n, T_t^{iz}(\psi)) = \bar{e}^{\alpha s} \cdot v_j(n, T_s^{iz}(\psi)). \qquad (5)$$

It should be remembered that s is generally a function of i, j, and z.

The equations (4) can now be written as,

$$v_i(n, \psi) = \max_{z \in Z_i} \{\rho_i^z(\alpha, \psi) + \sum_{j=1}^{N} p_{ij}^z \bar{e}^{\alpha s} v_j(n-1, T_s^{iz}(\psi))\} \qquad (6)$$

$i = 1, 2, \ldots, N.$

Note that

$$0 < \bar{e}^{s} < 1 \qquad (7)$$

for $i, j = 1, 2, \ldots, N; \quad z \in Z_{ij}$ and $\alpha < 0$.

The following 2 lemmas and 2 theorems establish the existance of an optimal solution to the MRD under consideration. They also show that the sequence of approximants, $\{v_i(n, \psi)\}$ converges to the optimal solution.

*Lemma 1*

If

$$V \geq |V_i(\psi)| \qquad (8)$$

$$R^* = \max_{i,z} \{ |\rho_i^z(\alpha, \psi)| \} \tag{9}$$

and

$$\beta = \max_{i,j,z} \{\bar{e}^{\alpha s}\}, \tag{10}$$

then

$$|v_i(n, \psi)| \leq \beta^n V + \frac{1-\beta^n}{1-\beta} R^* \tag{11}$$

for all $n \geq 0, i = 1, 2, \ldots, N$.

*Lemma 2*

Let V, R*, and $\beta$ be defined as for the previous lemma. Then, for all $m < n$

$$|v_i(n, \psi) - v_i(m, \psi)| \leq (\beta^n + \beta^m)V + \frac{\beta^m - \beta^n}{1-\beta} R^* \tag{12}$$

$i = 1, 2, \ldots, N; \quad n \geq 0.$

*Theorem 1*

Limit of the Sequence $\{v_i(n, \psi)\}$, as n approaches infinity, exists.

*Theorem 2*

Let

$$v^* = \min_i \{V_i(\psi)\}, \qquad V^* = \max_i \{V_i(\psi)\}$$

$$r^* = \min_{i,z} \{\rho_i^z(\alpha, \psi)\}, \qquad R^* = \max_{i,z} \{\rho_i^z(\alpha, \psi)\}$$

$$\beta' = \min_{i,j,z} \{\bar{e}^{\alpha s}\}, \qquad \beta'' = \max_{i,j,z} \{\bar{e}^{\alpha s}\}.$$

If $r^* + \beta' v^* \geq V^*$, then $\{v_i(n, \psi)\}$ increases monotonically to $\{v_i(\psi)\}$, and if $R^* + \beta'' V^* \leq v^*$, then $\{v_i(n, \psi)\}$ decreases monotonically to $\{v_i(\psi)\}$ as n increases $(i = 1, 2, \ldots, N)$.

The equations (4) can be used to arrive at the optimal solution by iterating on n. A stopping rule that guarantees an $\varepsilon$-optimal solution in a finite number of steps is based on the fact that the sequence

$$(\beta'')^n \left\{\max\left(\frac{R^*}{1-\beta''} - v^*, V^* - \frac{r^*}{1-\beta'}\right)\right\}$$

converges monotonically to zero.

If the transition interval can take only discrete values as in the example presented in the next section, then the frequency count can be used to estimate the

corresponding probability mass function. Let sample $I_n = \{i_0, i_1, i_2, \ldots, i_n\}$ of n transitions of the system be obtained by the consecutive sampling rule. Here $i_0$ is the initial state of the system known prior to the sampling. Let $X_n = \{x_1, x_2, \ldots, x_n\}$ be the transition intervals corresponding to $I_n$. When starting from State i and using the $z^{th}$ alternative, let $\nu_x^{iz}$ be the observed number of transitions of interval x. The matrix of transition counts, $\nu = \{\nu_x^{iz}\}$ is a Z x T matrix where $Z = \sum_{i=1}^{N} Z_i$ and T is the number of discrete values that the transition interval can assume.

Given that the prior probability mass function the transition interval is $\phi(t/\psi) = \{\phi_x^{iz}\}$, the conditional probability of observing $X_n$ is

$$\prod_{i=1}^{N} \prod_{z=1}^{Z_i} \prod_{x=1}^{T} (\phi_x^{iz})^{\nu_x^{iz}} \quad . \tag{13}$$

If the stopping rule that determined the sample size n is noninformative, then equation (13) is the likelihood of $X_n$. Hence $\phi(t/\psi) = \{\phi_x^{iz}\}$ has a matrix beta distribution with parameter $\psi$; $\psi = \{\psi_x^{iz}\}$ is a Z x T matrix, and

$$\psi_x^{iz} = \psi_x^{iz} + 1 \tag{14}$$

$$i = 1, 2, \ldots, N; \quad z = 1, 2, \ldots, Z_i; \quad x = 1, 2, \ldots, T.$$

It can be shown that if a sample $X_n$ is obtained from a population that has matrix beta prior distribution with parameter $\psi$, then the posterior distribution is also matrix beta with parameter $\psi + \nu$ where $\nu$ is the matrix of frequency counts resulting from $X_n$.

*Example of an MRD:* $\{P, \tilde{F}, R, Z\}$

Consider an MRD: $\{P, \tilde{F}, R, Z\}$ with 2 states and 2 alternatives in each state. The transition intervals can be either 1 or 2 units of time, the probability mass function governing it is now known. The matrix of frequency counts is

$$\nu = \{\nu_t^{iz}\} = \begin{bmatrix} 6 & 4 \\ 1 & 12 \\ 2 & 5 \\ 5 & 2 \end{bmatrix} .$$

from which $\psi$ is obtained to be

$$\psi = \{\psi_t^{iz}\} = \{\nu_t^{iz} + 1\} = \begin{bmatrix} 7 & 5 \\ 2 & 13 \\ 3 & 6 \\ 6 & 3 \end{bmatrix} .$$

The transition probabilities are:

$$P = \{p_{ij}^z\} = \begin{bmatrix} 0.5 & 0.5 \\ 0.8 & 0.2 \\ 0.4 & 0.6 \\ 0.8 & 0.2 \end{bmatrix}.$$

Now,

$$f_{ij}^z(t/\psi) = p_{ij}^z \cdot \psi_t^{iz}.$$

Hence

$$\{f_{ij}^z(t/\psi)\} = \begin{bmatrix} 0.29 & 0.21 & 0.29 & 0.21 \\ 0.11 & 0.69 & 0.03 & 0.17 \\ 0.13 & 0.27 & 0.20 & 0.40 \\ 0.53 & 0.27 & 0.13 & 0.07 \end{bmatrix}.$$

When the system is in State i and is being operated under the $z^{th}$ alternative, let the reward accumulated be $r_i^z$ units per unit of time,

$$R = \{r_i^z\} = \begin{bmatrix} 9 \\ 6 \\ -3 \\ -6 \end{bmatrix}.$$

The problem is to maximize the total expected discounted reward when the system is operated for an indefinitely long time. Continuous discounting, $\bar{e}^{\alpha t}$ with $\alpha = 2$, is to be used.

Now,

$$\rho_{ij}^z(\alpha, \psi) = \sum_{t=1}^{2} f_{ij}^z(t/\psi) \cdot \int_0^t \bar{e}^{\alpha x} \cdot r_i^z \cdot dx$$

$$= \frac{r_i^z}{\alpha} \{f_{ij}^z(1/\psi)(1-\bar{e}^{\alpha}) + f_{ij}^z(2/\psi)(1-\bar{e}^{2\alpha})\}.$$

Thus,

$$\{\rho_{ij}^z(\alpha, \psi)\} = \begin{bmatrix} 2.057 & 2.057 \\ 2.318 & 0.579 \\ -0.566 & -0.849 \\ -2.171 & -0.544 \end{bmatrix}$$

and

$$\{\rho_i^z(\alpha, \psi)\} = \begin{bmatrix} 2.057 \\ 1.970 \\ -0.736 \\ -1.846 \end{bmatrix}.$$

Assume a starting policy $\zeta(0, \psi) = \begin{bmatrix} 1 \\ 1 \end{bmatrix}$. Then,

$$v(0, \psi) = \begin{bmatrix} 2.057 \\ -0.736 \end{bmatrix}.$$

If alternative 1 is used when starting from State 1, then,

$$\begin{aligned} v_1^1(1, \psi) = \rho_1^1(\alpha, \psi) &+ p_{11}^1 \{f_{11}^1(1/\psi) \cdot \bar{e}^{\alpha} \cdot v_1(0, T_1^{11}(\psi)) \\ &+ f_{11}^1(2/\psi) \cdot \bar{e}^{2\alpha} \cdot v_1(0, T_2^{11}(\psi))\} \\ &+ p_{12}^1 f_{12}^1(1/\psi) \cdot \bar{e}^{\alpha} \cdot v_2(0, T_1^{11}(\psi)) \\ &+ f_{12}^1(2/\psi) \cdot \bar{e}^{2\alpha} \cdot v_2(0, T_2^{11}(\psi))\}. \end{aligned}$$

Similarly $v_1^2(1, \psi)$, $v_2^1(1, \psi)$, and $v_2^2(1, \psi)$ are computed. Then

$$v_1(1, \psi) = \max(v_1^1(1, \psi); v_1^2(1, \psi))$$

and

$$v_2(1, \psi) = \max(v_2^1(1, \psi); v_2^2(1, \psi)).$$

Next,

$$T_1^{11}(\psi) = \begin{bmatrix} 8 & 5 \\ 2 & 13 \\ 3 & 6 \\ 6 & 3 \end{bmatrix}$$

which yields

$$v(0, T_1^{11}(\psi)) = \begin{bmatrix} 2.046 \\ -0.736 \end{bmatrix}.$$

Similarly, $v(0, T_2^{11}(\psi)) = \begin{bmatrix} 2.085 \\ -0.736 \end{bmatrix}$, etc. Using these values, the next approximant is computed:

$$v(1, \psi) = \begin{bmatrix} 2.085 \\ -0.733 \end{bmatrix}, \qquad \zeta(1, \psi) = \begin{bmatrix} 1 \\ 1 \end{bmatrix}.$$

Repeating the iteration once more,

$$v(2, \psi) = \begin{bmatrix} 2.086 \\ -0.732 \end{bmatrix}, \qquad \zeta(2, \psi) = \begin{bmatrix} 1 \\ 1 \end{bmatrix}.$$

The results are presented in Table 1.

TABLE 1

Summary of Computations for the Example MRD: $\{P, \tilde{F}, R, Z\}$.

| n | $v(n, \psi)$ | $\zeta(n, \psi)$ |
|---|---|---|
| 0 | 2.057 | 1 |
| | -0.736 | 1 |
| 1 | 2.085 | 1 |
| | -0.733 | 1 |
| 2 | 2.086 | 1 |
| | -0.732 | 1 |

*Optimization Under Incomplete Information About Both P and F*

Consider and MRD: $\{\tilde{P}, \tilde{F}, R, Z\}$. Let the transition probability matrix, $\tilde{P} = \{\tilde{p}_{ij}^z\}$, have a distribution function $G(P/\mu)$ with parameter $\mu$. Let G belong to a family $\Gamma$ of distributions closed under consecutive sampling rule. Similarly, let the transition interval distribution, $\tilde{F} = \{\tilde{F}_{ij}^z(t/\psi)\}$ with parameter $\psi$, belong to a family $\Phi$ which is closed under consecutive sampling rule.

When the system starts from the state $(i, \mu, \psi)$, the expected discounted reward due to the transition from i to j under the $z^{th}$ alternative in i is,

$$\rho_{ij}^z(\alpha, \psi) = \int_0^\infty dF_{ij}^z(t/\psi) \cdot \int_0^t e^{\alpha x} \cdot d_x R_{ij}^z(x/t). \tag{15}$$

The expected 1-step reward is,

$$\rho_i^z(\alpha, \mu, \psi) = \sum_{j=1}^N \bar{p}_{ij}^z(\mu) \cdot \rho_{ij}^z(\alpha, \psi). \tag{16}$$

Let $v_i(\mu, \psi)$ be the maximum expected discounted reward that is accumulated if the system is operated over an indefinitely long period of time starting from State $(i, \mu, \psi)$. Then $v_i(\mu, \psi)$ must satisfy the following simultaneous functional equations:

$$v_i(\mu,\psi) = \max_{z \in Z_i} \{\rho_i^z(\alpha,\mu,\psi) + \sum_{j=1}^N \bar{p}_{ij}^z(\mu) \cdot \int_0^\infty dF_{ij}^z(t/\psi) \cdot e^{\alpha t} \cdot v_j(T_{ijt}^z(\mu,\psi))\}$$

$$i = 1, 2, \ldots, N; \quad \alpha > 0; \quad \mu \in M; \quad \psi \in \Psi \tag{17}$$

Here, again, $T_{ijt}^z(\mu, \psi)$ is the mapping of M into M and $\Psi$ into $\Psi$ due to the transition from i to j under the $z^{th}$ alternative in i in an interval of t units.

Define a sequence, $\{v_i(n, \mu, \psi)\}$, recursively as follows:

$$v_i(n,\mu,\psi) = \max_{z \in Z_i} \{\rho_i^z(\alpha,\mu,\psi) + \sum_{j=1}^N \bar{p}_{ij}^z(\mu) \cdot \int_0^\infty dF_{ij}^z(t/\psi) \cdot e^{\alpha t} \cdot v_j(n-1, T_{ijt}^z(\mu,\psi))\}$$

$$i = 1, 2, \ldots, N; \quad \alpha > 0 \tag{18}$$

Let the range of the transition intervals be $0 \leq t_1 < t_2 < \infty$. By mean value theorem, there is a value s such that $t_1 < s < t_2$, and

$$\int_{t_1}^{t_2} dF_{ij}^z(t/\psi) \cdot \bar{e}^{\alpha t} \cdot v_j(n, T_{ijt}^z(\mu, \psi)) = \bar{e}^{\alpha s} \cdot v_j(n, T_{ijs}^z(\mu, \psi)) \cdot \int_{t_1}^{t_2} dF_{ij}^z(t/\psi).$$

That is,

$$\int_{t_1}^{t_2} dF_{ij}^z(t/\psi) \cdot \bar{e}^{\alpha t} \cdot v_j(n, T_{ijt}^z(\mu, \psi)) = \bar{e}^{\alpha s} \cdot v_j(n, T_{ijs}^z(\mu, \psi)). \tag{19}$$

Thus, equations (18) can be rewritten as,

$$v_i(n, \mu, \psi) = \max_{z \in Z_i} \{\rho_i^z(\alpha, \mu, \psi) + \sum_{j=1}^{N} \bar{p}_{ij}^z(\mu) \cdot \bar{e}^{\alpha s} \cdot v_j(n, T_{ijs}^z(\mu, \psi))\} \tag{20}$$

$i = 1, 2, \ldots, N.$

*Lemma 3*

If

$$V \geq |V_i(\mu, \psi)| \tag{21}$$

$$R^* = \max_{i,z} \{|\rho_i^z(\alpha, \mu, \psi)|\}, \tag{22}$$

$$\beta = \max_{i,j,z} \{\bar{e}^{\alpha s}\}, \tag{23}$$

then

$$|v_i(n, \mu, \psi)\} \leq \beta^n V + \frac{1-\beta^n}{1-\beta} R^* \tag{24}$$

for all $n \geq 0, i = 1, 2, \ldots, N.$

*Lemma 4*

If V, R*, and $\beta$ are defined as for the previous lemma, then for all $m < n$,

$$|v_i(n, \mu, \psi) - v_i(m, \mu, \psi)| \leq (\beta^n + \beta^m)V + \frac{\beta^m - \beta^n}{1-\beta} R^* \tag{25}$$

for all $n \geq 0, i = 1, 2, \ldots, N.$

*Theorem 3*

The limit of the sequence $\{v_i(n, \mu, \psi)\}$, as n approaches infinity, exists.

*Theorem 4*

Let

$$v^* = \min_i \{V_i(\mu, \psi)\}, \qquad V^* = \max_i \{V_i(\mu, \psi)\},$$

$$r^* = \min_{i,z}\{\rho_i^z(\alpha,\mu,\psi)\}, \qquad R^* = \max_{i,z}\{\rho_i^z(\alpha,\mu,\psi)\},$$

$$\beta' = \min_{i,j,z}\{\tilde{e}^{\alpha s}\}, \qquad \beta'' = \max_{i,j,z}\{\tilde{e}^{\alpha s}\}.$$

If $r^* + \beta' v^* \geq V^*$, then $\{v_i(n,\mu,\psi)\}$ increases monotonically to $\{v_i(\mu,\psi)\}$, and if $R^* + \beta'' V^* \leq v^*$, then $\{v_i(n,\mu,\psi)\}$ decreases monotonically to $\{v_i(\mu,\psi)\}$ as n increases ($i = 1, 2, \ldots, N$).

The preceding 2 lemmas and the 2 theorems establish that the sequence of approximants, $\{v_i(n,\mu,\psi)\}$, converges to the optimal solution $\{v_i(\mu,\psi)\}$. Theorem 4 also establishes a set of sufficient conditions for the convergence of that sequence to be monotonic.

The equations (18) can be used iteratively to arrive at the optimal or an acceptable solution. The number of iterations, n, required to guarantee a prescribed $\varepsilon$-optimality may be computed from

$$(\beta'')^n \max\left\{\frac{R^*}{1-\beta''} - v^*;\quad V^* - \frac{r^*}{1-\beta'}\right\} < \varepsilon \tag{26}$$

*Example of an MRD:* $\{\tilde{P}, \tilde{F}, R, Z\}$

Consider an MRD: $\{\tilde{P}, \tilde{F}, R, Z\}$ with 2 states and 2 alternatives in each state. The transitions can take place in either one or two units of time with unknown probability. A matrix of frequency counts from such a system is given below:

| | | | State 1 Trans. Interval 1 unit | State 1 Trans. Interval 2 units | State 2 Trans. Interval 1 unit | State 2 Trans. Interval 2 units |
|---|---|---|---|---|---|---|
| $\nu =$ | State 1 | $z = 1$ | 7 | 3 | 5 | 5 |
| | | $z = 2$ | 1 | 21 | 1 | 3 |
| | State 2 | $z = 1$ | 1 | 4 | 2 | 7 |
| | | $z = 2$ | 13 | 6 | 3 | 1 |

From this matrix, $\bar{P}(\mu)$ and $\{f_{ij}^z(t/\psi)\}$ are calculated to be

$$\bar{P}(\mu) = \begin{bmatrix} 0.50 & 0.50 \\ 0.80 & 0.20 \\ 0.39 & 0.61 \\ 0.78 & 0.22 \end{bmatrix}$$

$$\{f_{ij}^z(t/\psi)\} = \begin{bmatrix} 0.33 & 0.17 & 0.25 & 0.25 \\ 0.07 & 0.73 & 0.07 & 0.13 \\ 0.11 & 0.28 & 0.17 & 0.44 \\ 0.52 & 0.26 & 0.15 & 0.07 \end{bmatrix}$$

When the system is in State i and is being operated under the $z^{th}$ alternative, let the reward be $r_i^z$ units per unit time, with,

$$\{r_i^z\} = \begin{bmatrix} 9 \\ 6 \\ -3 \\ -6 \end{bmatrix}.$$

The problem is to determine the maximum expected discounted reward that is accumulated from operating the system for an indefinitely long period. Continuous discounting with $\alpha = 2$ is to be used.

Now,

$$\begin{aligned} f_{ij}^z(\alpha, \psi) &= \int_0^\infty dF_{ij}^z(t/\psi) \cdot \int_0^t \bar{e}^{\alpha x} \cdot r_i^z \cdot d_x \\ &= \frac{r_i^z}{\alpha} f_{ij}^z(1/\psi)(1-\bar{e}^{\alpha}) + f_{ij}^z(2/\psi)(1-\bar{e}^{2\alpha}). \end{aligned}$$

Therefore,

$$\{\rho_{ij}^z(\alpha, \psi)\} = \begin{bmatrix} 2.036 & 2.078 \\ 2.332 & 0.565 \\ -0.555 & -0.869 \\ -2.115 & 0.595 \end{bmatrix}$$

and,

$$\rho_i^z(\alpha, \mu, \psi) = \sum_{j=1}^{N} \bar{p}_{ij}^z(\mu) \cdot \rho_{ij}^z(\alpha, \psi),$$

hence

$$\{\rho_i^z(\alpha, \mu, \psi)\} = \begin{bmatrix} 2.057 \\ 1.979 \\ -0.738 \\ -0.811 \end{bmatrix}.$$

Assume

$$\zeta(0, \mu, \psi) = \begin{bmatrix} 1 \\ 1 \end{bmatrix}.$$

Therefore,

$$v(0, \mu, \psi) = \begin{bmatrix} 2.057 \\ -0.738 \end{bmatrix}.$$

Next,

$$v_i(1,\mu,\psi) = \max_{z=1,2} \{\rho_i^z(\alpha,\mu,\psi) + \bar{p}_{11}^z(\mu)[f_{11}^z(1/\psi)\cdot\bar{e}^{\alpha}\cdot v_1(0,T_{111}^z(\mu,\psi))$$
$$+ f_{11}^z(2/\psi)\cdot\bar{e}^{2\alpha}\cdot v_1(0,T_{112}^z(\mu,\psi))]$$
$$+ \bar{p}_{12}^z(\mu)[f_{12}^z(1/\psi)\cdot\bar{e}^{\alpha}\cdot v_2(0,T_{121}^z(\mu,\psi))$$
$$+ f_{12}^z(2/\psi)\cdot\bar{e}^{2\alpha}\cdot v_2(0,T_{122}^z(\mu,\psi))]\}.$$

From this, $v_1(1,\mu,\psi)$ was found to be 2.092. Similarly, $v_2(1,\mu,\psi)$ was found to be -0.736. The calculations were performed for the next successive approximants and the results are presented in Table 2.

TABLE 2

Summary of Computations for the Example MRD: $\{\widetilde{P}, \widetilde{F}, R, Z\}$.

| n | $v(n,\mu,\psi)$ | $\zeta(n,\mu,\psi)$ |
|---|---|---|
| 0 | 2.057 | 1 |
| | -0.738 | 1 |
| 1 | 2.092 | 1 |
| | -0.736 | 1 |
| 2 | 2.092 | 1 |
| | -0.736 | 1 |

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# SOME COMMENTS CONCERNING THE IMPACT BALL APPARATUS

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A typical impact ball apparatus consisting of an arbitrary number of balls *in contact* is illustrated in Figure 1. The usual demonstration is to pull one or more balls aside, maintaining contact between them, and then to allow these to collide with the remaining spheres. Despite previous discussions in the literature (Chapman, 1960; Lemon, 1935; Schilling and Yeagley, 1947; Walkiewicz and Newby, 1972), this apparatus is still misunderstood by many teachers and authors. It is the purpose of this paper to analyze the simplest case so that physics teachers can give their students a simple, yet accurate, interpretation of the results.

Quite often the statement is made that a simple application of the principles of conservation of momentum and conservation of energy are sufficient to define the motion immediately after the collision. This statement is incorrect. Furthermore, when the motion is allowed to continue for a few collisions in a "symmetric" fashion, the observation that things get "messed-up" is usually blamed on the suspension system. This explanation, at best, is misleading. These explanations fail to touch on the central point necessary to understand the demonstration.

The case to be examined is shown in Figure 2. The spheres are designated 1, 2, 3, left to right. The positive direction is to the right. Sphere 1 has an initial positive speed $v_{1i}$ just before impact. As shall be discussed, the principles of conservation of energy and momentum do not guarantee that the speed of Sphere 1 will be zero after the impact. Its speed after the collision will be designated $v_{1f}$. Other speeds are similarly designated. The initial conditions are $v_{2i} = v_{3i} = 0$. Application of the conservation conditions and elimination of $v_{1i}$ immediately leads to

$$v_{1f} = -\frac{(v_{2f})(v_{3f})}{v_{2f} + v_{3f}}. \tag{1}$$

From this relation, the usual observation is made, i.e., if $v_{1f} = 0$ then either $v_{2f}$ or $v_{3f}$ must be zero. The constraint that one ball not pass through the other then dictates that $v_{2f} = 0$. The result that $v_{3f} = v_{1i}$ then follows. However, this is not the only solution since positive and negative values of $v_{1f}$ should be considered. Without loss of generality, the magnitude of $v_{1f}$ can be taken to be unity. Then

$$v_{2f} = -\frac{v_{3f}}{1 + v_{3f}} \qquad \text{Case A, } v_{1f} = +1 \tag{2}$$

$$v_{2f} = -\frac{v_{3f}}{1 - v_{3f}} \qquad \text{Case B, } v_{1f} = -1 \tag{3}$$

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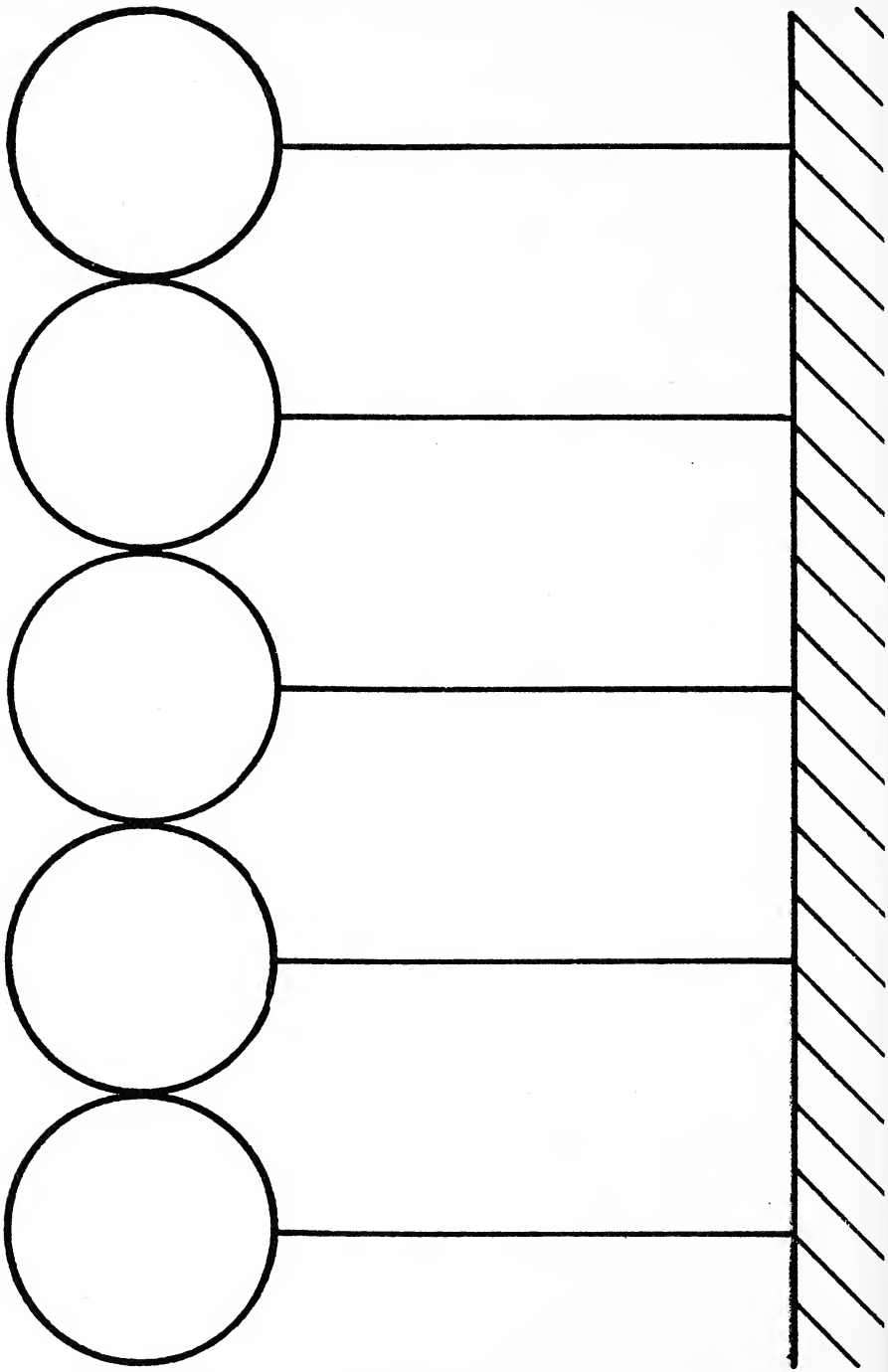

Figure 1. Schematic drawing of the impact ball apparatus. Any number of balls may be used.

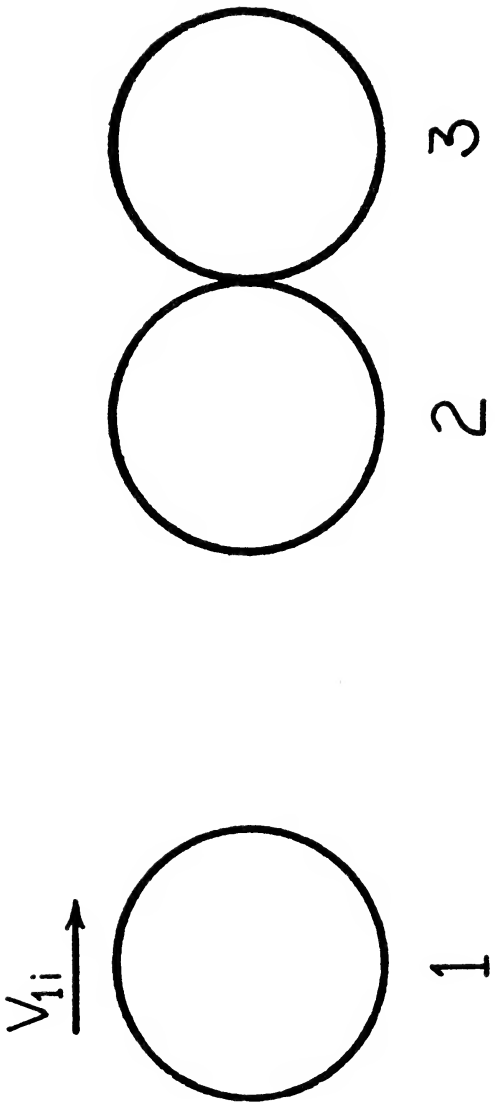


Figure 2. A head-on perfectly elastic collision in which Ball 1 impacts Ball 2. Balls 2 and 3 are at rest and in contact before the collision.

The graphs for these hyperbolic functions are shown in Figures 3 and 4. The dashed lines indicate regions of solutions to the equations which do not represent permissable motion. In Case A, none of the solutions are permissable since, if $v_{1f}$ is positive, *both* $v_{2f}$ and $v_{3f}$ must be positive or Ball 1 would pass through the other two. In Case B, the regions indicated are not permitted for the following reasons:

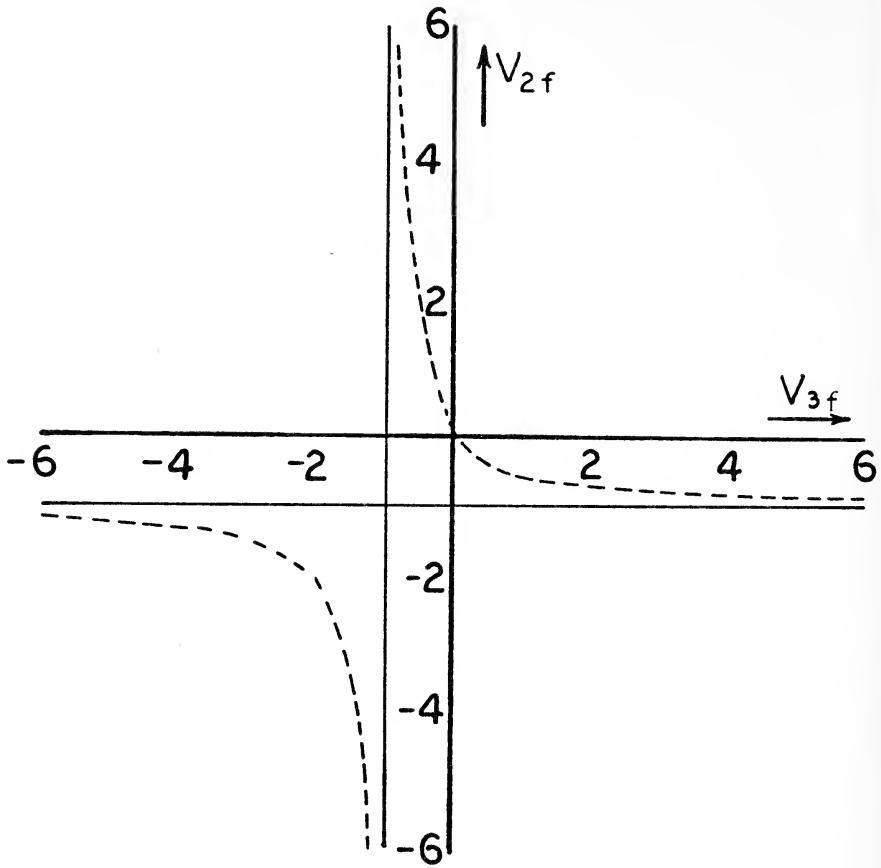


Figure 3. Graph of Equation 2, corresponding to $v_{1f} = +1$. This motion cannot occur.

Quadrant I - Since Ball 2 cannot pass through Ball 3, we require $v_{3f} \geq v_{2f}$.

Quadrant II - Since $v_{1f}$ is negative, $v_{3f}$ cannot also be negative since this would mean that all 3 balls would be traveling in the negative direction after the collision.

Quadrant III - Since $v_{1f}$ is negative, $v_{2f}$ can only be negative if $v_{3f}$ is positive and $v_{3f} > v_{2f}$ such that the net momentum will be positive after the collision, no point on the curve satisfies this condition.

It is reasonable to ask if one should have anticipated that $v_{1f}$ could not be positive. The answer can probably be deduced quite simply by considering the head-on collision of a ball of mass m with another at rest whose mass is m'. The conservation principles show that the ball of mass m will rebound if $m' > m$. In the three-ball case considered here, one would expect $m_2$ to behave as if its mass were slightly greater than $m_1$ due to the influence of $m_3$. Thus, rebound of $m_1$ is to be expected.

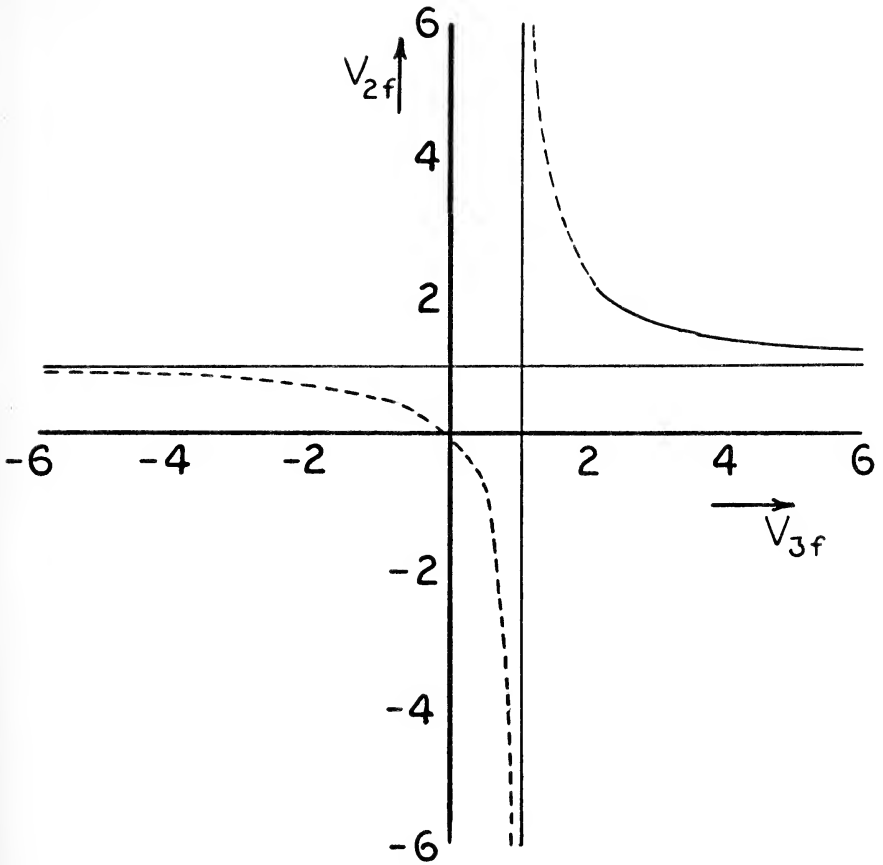


Figure 4. Graph of Equation 3, corresponding to $v_{1f} = -1$. Only the solid-line branch represents permitted motion.

The motion observed when the demonstration is performed is that both $v_{1f}$ and $v_{2f}$ are small. The experiments and calculations of Chapman (1960) show that the only cases which seem to occur are those for which $v_{1f}$ is small and negative. Chapman modeled the problem by imagining the spheres to be separated by massless springs, thus invoking a Hooke's Law interaction force. The results were calculated to be $v_{1f} = -0.1303\, v_{1i}$, $v_{2f} = +0.1503\, v_{1i}$, and $v_{2f} = +0.9800\, v_{1i}$. Chapman also calculated results using a 3/2 power interaction. The results were qualitatively the same as for Hooke's Law. In an experiment involving steel balls, the results were close to the calculated values.

The application of the principles of conservation of energy and momentum to the problem of a perfectly elastic collision as indicated in Figure 2 is thus seen to permit a variety of motions after the collision. These motions are limited to $v_{1f} < 0$, $v_{2f} > 0$, and $v_{3f} > 0$. These solutions are, of course, in addition to the single case

$v_{1f} = v_{2f} = 0$, $v_{3f} = v_{1i}$. In order to uniquely predict the motion, a specific force law must be assumed. The only case observed to occur, however, is in the asymptotic region of Figure 4 corresponding to $v_{3f}$ being large.

Since this problem is often misunderstood, it can be instructive to ask students to analyze it on their own. This suggestion is also made by Walkiewicz and Newby (1972), but the method of analysis they use, though of a more general nature, requires more insight on the part of the student. A simple method for giving the assignment would be to have them derive Equation 1 and then discuss the physically acceptable motion consistent with the equation.

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# PETROGRAPHY OF PLEISTOCENE VOLCANIC ASH FROM PALO DURO CANYON, TEXAS

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ABSTRACT

Samples from several Palo Duro Canyon volcanic ash outcrops were examined petrographically and particle size distributions were determined through dry-sieving and pipetting. The examined properties of the glass and the heavy minerals indicate that these ash lenses correlate with the Pearlette-like ash type O as defined by Izett,*et al.*

## INTRODUCTION

It was the purpose of this study to identify, petrographically, several Palo Duro Canyon volcanic ashes and to correlate them with better known ash beds. On the basis of stratigraphic position, megascopic resemblance, underlying mollusk fauna (Johnston and Savage, 1955) and micro-vertebrate fauna (Schult, personal communication, 1970), the local ash lenses have been considered to be Pearlette ash. To determine the validity of this assumption, a petrographic examination of a Pearlette ash sample from the Borchers locality in Meade County, Kansas, was included in this study. To complement the petrographic findings, grain size distributions were determined for each ash sample through dry sieving and pipetting.

## ASH LOCATIONS

### *Bull Draw Ash Locality*

The main exposure is approximately 50 ft long, 3½ ft thick, and is located on the west side of Bull Draw, ½ mi above its junction with North Cita Creek. Sample 68L38 was collected from the lower 6 in of the main exposure, while sample 68L39 was collected from the upper 6 in of the outcrop.

### *Woody Draw Ash Locality*

The ash is exposed in several small canyons near the head of Woody Draw, a tributary of North Cita Creek. Sample 68L42 was collected from the lower 6 in of the 2½ ft thick ash bed.

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*Sunday Creek Ash Locality*

The main outcrop is 8½ ft thick and is located near the head of a north flowing tributary of Sunday Creek. Sample 68L43 was collected from the lower 6 in of the bed.

Sample 68L44 was collected about 750 ft WNW of locality 68L43 on the other side of the interfluve, 2-3 ft above the base of the ashbed which is about 10 ft thick at this locality.

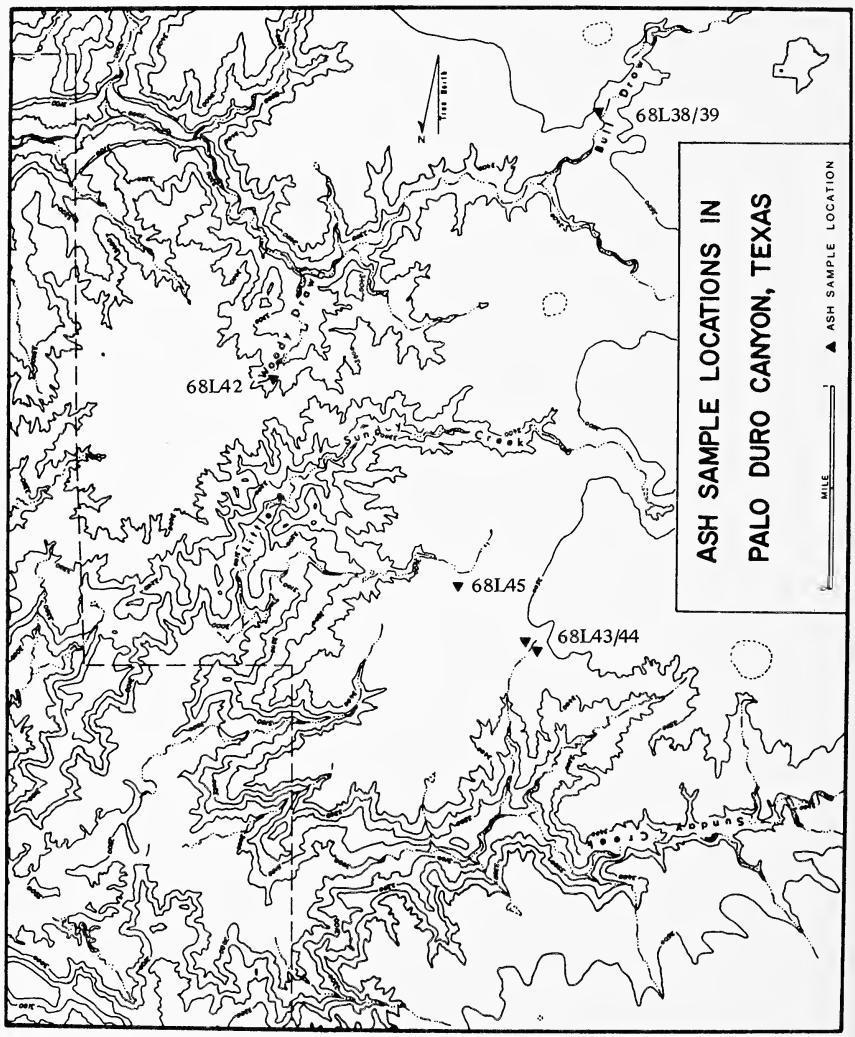



Figure 1. Ash sample locations in Palo Duro Canyon, Texas.

*Little Sunday Creek Ash Locality*

The ash is exposed near the head of an east flowing tributary of Little Sunday Creek. The sample site is on the east side of a north flowing arroyo which drains into the tributary. Sample 68L45 was collected 2 ft above the base of the 2-5 ft thick bed.

All of the above ash locations are in Randall County, Texas, and are shown in Figure 1.

## LABORATORY PROCEDURE

Approximately 400 g of each ash sample were carefully desegregated and scrubbed ultrasonically in water for 5 min and decanted 5 times after settling periods of 60 sec. The decanted materials were filtered, dried and studied. The remaining slurries were poured into large evaporating dishes and dried in a heat chamber for 2 or more hours.

Before heavy liquid separation, the magnetic fraction was removed by magnet. The non-magnetic fraction was separated into glass and 2.42+ SG fractions using a Bromoform-Acetone mixture with a specific gravity of 2.42. The samples were stirred for 3 min in separatory funnels containing the 2.42 SG Bromoform-Acetone mixture and the ash. After settling periods of 10 min the heavier fractions were tapped off. This stirring, settling, and tapping process was repeated 3-5 times per separation. Both, the glass and the 2.42+ SG heavies were rinsed with acetone and dried.

The 2.42+ fraction was again separated into a 2.42-2.8 fraction and a 2.8+ fraction using pure Bromoform with a specific gravity of 2.8. These separating processes were the same as described above.

The indices of refraction of the glass were determined by Becke line and oblique illumination methods. Due to the inavailability of high dispersion liquids, the more accurate focal masking technique was not used. Glass shards were examined in form of immersion mounts using microscope slides, oil of known index and no. 0 cover glasses. All determinations were made with white light and repeated with a sodium filter (594 nm) placed over the light source. A thermometer was attached to the microscope stage and readings were taken before and after each determination in order to compensate for the refractive index change of the oils due to tempaerature changes.

The shards were studied by traversing the slide on the mechanical stage, identifying and counting each grain that passed under the crosshair. A minimum of 300 grains was counted for each slide. Each shard was identified as to shard type and index of refraction. The indices were recorded relative to the oil: $n_{glass} < n_{oil}$, $n_{glass} = n_{oil}$, or $n_{glass} > n_{oil}$.

In smaller grains, it was often difficult to make rapid distinctions between glass shards and quartz or feldspar, but between crossed nicols and with the gypsum plate inserted, the separation was made easy by the red color of the

isotropic shards which contrasted sharply with the yellow and blue interference colors of quartz and feldspar. This method also made more prominent the fine-grained alteration products that coat the glass shards.

Using a Bausch and Lomb binocular microscope, the heavy minerals were examined and prepared for spindle stage study. The Wilcox spindle stage was used to identify the minerals and to determine their indices. For minerals with variable indices like Amphiboles and Pyroxenes, $n_x$ was recorded, otherwise the minerals were recorded by name only.

Heavy minerals were selected for spindle stage study only if they had glass adhering to them and if there was no doubt that they were of volcanic, and not of detrital origin. The index of the adhering glass was also determined to make sure it matched the index of the other glass shards. The petrographic work was done with both Leitz and Zeiss polarizing research microscopes.

For the grain size analysis, fresh ash samples were used. About 50-100 g of each ash sample were desegregated in an ultrasonic waterbath and oven dried. Each sample was dry- sieved through ¼ $\phi$ mesh sieves and a Rotap mechanical shaker. The 44$\mu$ fraction was further subdivided through pipetting into ½ $\phi$ fractions. (Galehouse, 1971).

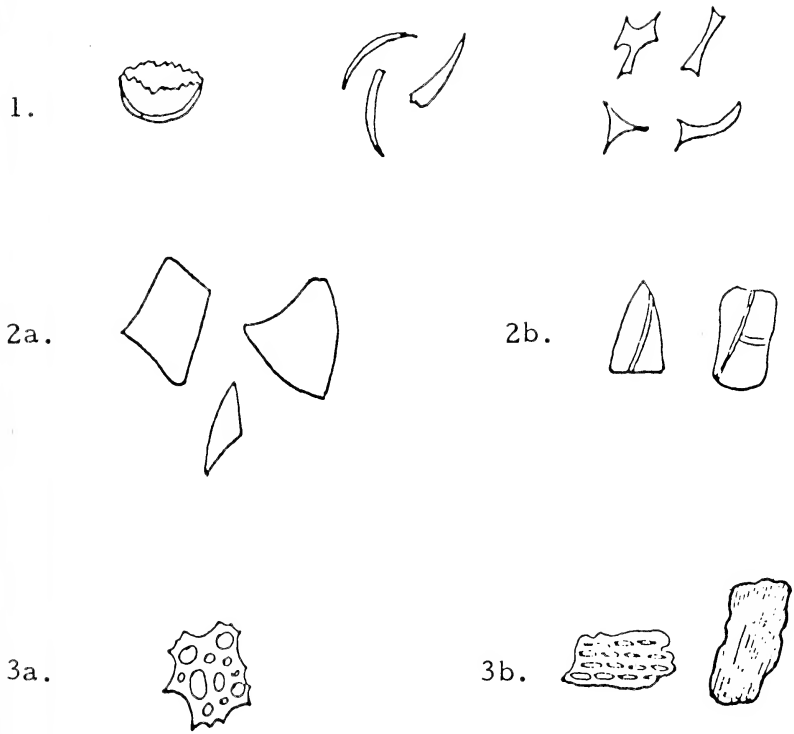
## GLASS

Megascopically, all glass fractions are silver-gray in color, while microscopically they appear to be somewhat pink under plain light. Few shards show any alterations, and in some cases, what appears to be alterations may be clay adhering to the shard. Under the microscope several kinds of glass shards can be distinguished (Figure 2). The most common ones are plain plates and plates with one or two ridges, which represent bubble walls and bubble junctions. Quite common are pumice fragments with elongate vesicles, while pumice fragments with spherical vesicles and spherical vesicle fragments are not very common in the Palo Duro Canyon ash. The indices of refraction range from 1.499 to 1.501 with the majority of the shards having an index of 1.500 (Table 1).

The decanted material consists mainly of clay, very small shards, and unbroken glass bubbles, with refractive indices matching those of the shards (Table 2). The bubbles float and are normally decanted during the cleaning cycle. It is apparently for this reason, that they have not been reported before.

## HEAVY MINERALS

The ash samples 68L38, 68L42, 68L43, and 68L44 contain clinopyroxene (ferroaugite, $n_x = 1.730 \pm .005$) and hornblende ($n_x = 1.692 \pm .002$) as diagnostic minerals, which have not been found in the Borcher's ash 67L16.




1. Spherical vesicles and vesicle fragments.
2a. Plain plates.
2b. Plates with one or two ridges (connecting walls).
3a. Pumice fragments with spherical vesicles.
3b. Pumice fragments, fibrous or with elongate vesicles.

Figure 2. Major glass shard types. Modified from Pirsson, 1915.

Chevkinite, allanite, quartz, plagioclase, sanidine, and colorless zircon have been found in all samples, while pink zircon was found only in the Borcher's ash and the Palo Duro ash 68L45. No hornblende was found in sample 68L45.

The most common detrital minerals, which are more or less abundant in all samples, are epidote, quartz, plagioclase, alkali feldspar, almandine garnet, hematite, metamorphic hornblende, and augite.

## PARTICLE SIZE DISTRIBUTION

As no fresh ash sample was available for the particle size analysis of the Borchers ash, the size analysis of this ash was performed on decanted remains

TABLE 1

Indices of Refraction and Shard Types of Glass.

| | | Shard types-% | | | |
|---|---|---|---|---|---|
| | | Plates | | Pumice fragments | |
| Sample No. | Index of refraction | plain | 1 or 2 ridges | fibrous or elongate vesicles | spherical vesicles |
| 68L38 | 1.500 ± .001 | 50 | 38 | 12 | <.1 |
| 68L39 | 1.500 ± .001 | 48 | 40 | 12 | <.1 |
| 68L42 | 1.500 ± .001 | 47 | 40 | 13 | <.1 |
| 68L43 | 1.500 ± .001 | 47 | 36 | 17 | <.1 |
| 68L44 | 1.500 ± .001 | 45 | 40 | 15 | <.1 |
| 68L45 | 1.500 ± .001 | 47 | 38 | 15 | <.1 |
| 67L16 | 1.500 ± .001 | 48 | 42 | 10 | <.1 |

TABLE 2

Heavy Minerals.

| Sample No. | Clino-pyroxene $n_x = 1.730 \pm .005$ | Horn-blende $n_x = 1.692 \pm .002$ | Zircon color-less | Zircon pink | Allan-ite | Chev-kinite | Plagio-clase | Quartz | Sani-dine |
|---|---|---|---|---|---|---|---|---|---|
| 68L38 | x | x | x | x | x | x | x | x | x |
| 68L39 | x | x | x | | x | x | x | x | x |
| 68L42 | x | x | x | | x | x | x | x | x |
| 68L43 | x | x | x | | x | x | x | x | x |
| 68L44 | x | x | x | | x | x | x | x | x |
| 68L45 | x | | x | x | x | x | x | x | x |
| 67L16 | | | x | x | x | x | x | x | x |

x = present

from earlier experiments. Consequently, the very fine fractions of this sample are missing and the percentages are skewed toward the larger grain sizes. Considering the absence of the fine portion of this sample (67L16), the particle size distribution curve is in fairly good agreement with those of the Palo Duro ashes.

Table 3 shows the distribtuion of particle sizes by percentage, while Figure 3 shows the relationship of the various size distribution curves to each other. The loss in sieving was reasonably small (less than 1½ g per sample) and was not included in the percentages.

TABLE 3

Particle Size Distribution by % of Weight.

| Size μ | 68L38 (%-wt) | 68L39 (%-wt) | 68L42 (%-wt) | 68L43 (%-wt) | 68L44 (%-wt) | 68L45 (%-wt) | 67L16 (%-wt) |
|---|---|---|---|---|---|---|---|
| 420 | 1.43 | 1.28 | .17 | .10 | .07 | .41 | 2.40 |
| 354 | .33 | .17 | .15 | .20 | .07 | .15 | 2.07 |
| 297 | .33 | .27 | .09 | .28 | .20 | .31 | 3.35 |
| 250 | .53 | .34 | .48 | .10 | .76 | .39 | 5.59 |
| 210 | 1.37 | .84 | .83 | .18 | 2.18 | .72 | 8.50 |
| 177 | .20 | .57 | .63 | .10 | 3.34 | .75 | 9.50 |
| 149 | .82 | 1.42 | 2.17 | .26 | 7.29 | 1.47 | 12.74 |
| 125 | .86 | 1.42 | .39 | .42 | 3.80 | .83 | 9.78 |
| 105 | 4.81 | 5.12 | 4.74 | 2.72 | 10.45 | 2.89 | 14.20 |
| 88 | 7.66 | 7.62 | 7.61 | 5.69 | 10.71 | 4.85 | 9.50 |
| 74 | 11.85 | 12.17 | 12.26 | 9.82 | 14.25 | 10.78 | 9.33 |
| 63 | 5.71 | 6.47 | 5.20 | 5.00 | 6.02 | 5.93 | 3.75 |
| 53 | 10.61 | 12.00 | 10.33 | 9.40 | 10.45 | 12.74 | 4.92 |
| 44 | 14.93 | 16.22 | 14.96 | 14.31 | 11.24 | 18.29 | 4.36 |
| 31 | 14.13 | 13.86 | 15.91 | 9.76 | 13.64 | 16.28 | - |
| 22 | 14.66 | 15.17 | 17.59 | 15.87 | 2.77 | 16.77 | - |
| 16 | 5.86 | 3.37 | 6.48 | 12.90 | 2.77 | 6.45 | - |
| 16 | 3.91 | 1.69 | - | 12.90 | - | - | - |
| | 100.00 | 100.00 | 99.99 | 100.01 | 100.01 | 100.01 | 99.99 |

## CONCLUSIONS

On the basis of petrographic and chemical data, Izett, *et al.*, (1970, 1971) informally classified Pearlette and Pearlette-like ash into types B, S, and O.

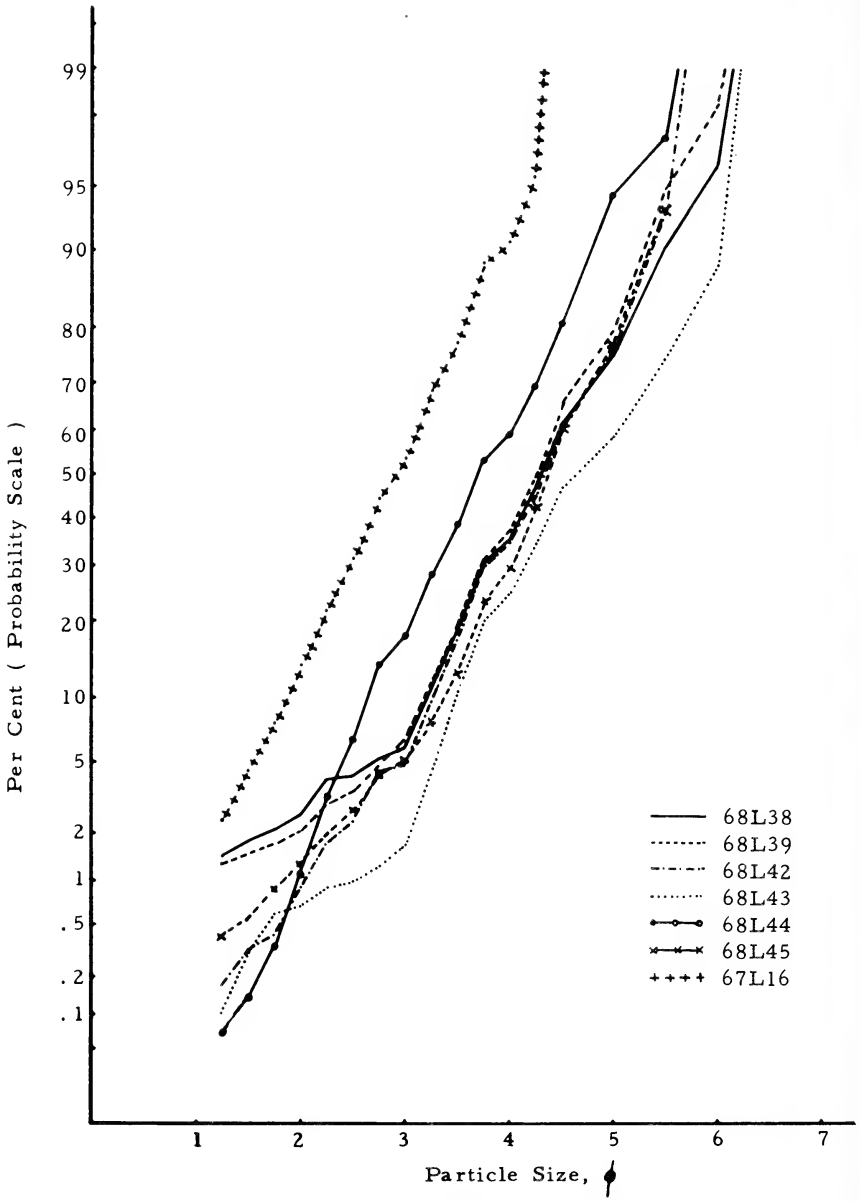



Figure 3. Relationship of the various size distribution cures to each other.

The type O ash is characterized by the presence of hornblende ($n_x$= 1.688-1.694; ave. 1.690) and clinopyroxene ($n_x$= 1.725-1.734; avg. 1.730). The Palo Duro ashes also contain hornblende ($n_x$= 1.692) and clinopyroxene ($n_x$= 1.730), and the petrographic properties of the glass fractions are also

identical to those of the type O ash. Therefore, the Palo Duro ashes correlate quite well with Izett's type O ash. The Verdos ash of Colorado (Scott, 1963), Harpole Mesa ash, Upper Onion Creek ash of Utah (Richmond, 1962), and Cudahy ash of Kansas (Frye, Swineford, and Leonard; 1948) are among the ash deposits that have been classified as type O (Lambert, personal communication, 1970).

The Palo Duro ashes differ greatly from the Borcher's ash of Kansas. While all Palo Duro ashes contain clinopyroxene, and all except sample 68L45 contain hornblende, the Borcher's ash contains neither clinopyroxene nor hornblende, Izett and Wilcox (Izett, personal communication, 1970) have also examined Borcher's ash samples and noted the absence of clinopyroxene and hornblende. Therefore, it seems very unlikely that additional ash separations and heavy mineral studies will reveal any of these critical minerals in the Borcher's ash.

The Palo Duro ashes are also very different from the Bishop ash and from type S ash. The Bishop ash's most diagnostic features are its chalky white color. The predominance of minutely pumiceous glass shards with indices ranging from 1.494-1.496, and the presence of biotite. In comparison, the Palo Duro ashes are silver-gray in color with bubble walls and junctions as major glass shards and with higher indices (1.499-1.501) than those of the Bishop ash. The Palo Duro ashes do not contain any biotite, and even the glass chemistry comparisons show that they are different from the Bishop ash.

The type S ash is distinguishable from the Palo Duro ashes by comparison of the refractive indices of hornblende, ($n_x$ = 1.680 for type S and $n_x$ = 1.692 for Palo Duro). The indices of clinopyroxene differ also ($n_x$ = 1.720 for type S, $n_x$ = 1.730 for Palo Duro), but more work is needed with clinopyroxenes before they can be used conclusively. The chemistry of the glass is also a good criterion for distinguishing Palo Duro ash from type S ash (Izett, personal communication, 1970).

The absence of hornblende in the sample 68L45 is most likely due to an insufficient size of sample, and it is expected that hornblende will be found in additional separations.

The Palo Duro ash has not yet been dated, but K-Ar dates on sanidine of several type O ashes indicate an age of about 0.6 million years.

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# APPLICATION OF HUYGENS' PRINCIPLE TO THE REFLECTION OF SEISMIC WAVES AT A FREE SURFACE

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ABSTRACT

This paper deals with the problem of a time-domain solution of a reflected spherical wave from a free surface. It is a study of the properties of the coefficients of the wave functions of the reflected compressional and shear waves in Kirchhoff's surface integral, developed by application of Huygens' Principle to a plane wave approximation of the velocity potentials. A technique for location of the secondary source in the form of equal-time rings on the reflecting surface is developed. The values of the coefficients of the first derivatives in the integral are insignificant as compared with those of the second derivatives, for both waves. The larger magnitudes occur immediately after reflection, and the largest when the observation point and the source point are on the same vertical. In this case the time rings are concentric circles with center and reflection point at the origin. When the observation point is near the reflecting surface and the origin, the magnitudes of the compressional wave coefficients are greater than those of the shear wave. As the observation point is moved away from the origin and the vertical, the magnitudes of the shear wave coefficients are greater than those of the compressional wave.

## INTRODUCTION

The purpose of this study has been the application of Huygens' Principle by use of Kirchhoff's integral to a plane wave approximation of compressional wave on a stress free surface.

In general terms Huygens' Principle states that if a source 0 is surrounded by a closed surface S, the various points of the surface S, when reached by the wave of the disturbance, become the origin of secondary waves, and the disturbance observed beyond the surface S results from the super-position of these secondary waves (Rossi, 1959). The Principle states that the disturbance at some later time, $t = t_0 + \Delta t$, at a given observation point, can be obtained by considering the effect produced by each point on a wave surface at a given time, $t = t_0$, acting as a secondary source (Officer, 1958).

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Huygens published his geometrical theory of wave propagation in optics, based on an analogy between the propagation of sound waves of small amplitude and the propagation of light, in 1690. Erroneously, he treated a light wave as a scalar phenomenon. Much of the work in his Treatise was vague, provoking a controversy which has lasted for may years and resulted in a wealth of published work on wave propagation.

A solution of the problem of transient elastic wave propagation through use of the Laplace transform was developed by Cagniard (1930). The transform methods give an answer, but not in the case of non-horizontal layers, nor do they give an understanding of the mechanism involved. Use of Kirchhoff's formula in the application of Huygens' Principle gives one a greater grasp of the physics of the problem.

Burridge (1963) made an exhaustive study of the reflection of a pulse in a solid sphere, using Kirchhoff's formula, and defined the pulse shape.

The application of Huygens' Principle to problems of elastic diffraction, using Kirchhoff's formula, has been investigated recently by Trorey (1970). He treated only the compressional wave.

This work is composed of 3 parts:

1. Development of the coefficients of the wave functions through substitution of given boundary conditions in Kirchhoff's surface integral (Baker and Copson, 1939).
2. Location of the secondary source reflecting surface, S, in the form of equal-time rings, for any given observation point within the medium bounded by S, but not on S.
3. Mapping of the secondary source rings and the values of the coefficients of the wave functions as time passes, for any given observation point, depth of source point, and velocity ratio; with numerical examples for varying positions of the observation point.

## COEFFICIENTS OF THE WAVE FUNCTIONS IN KIRCHHOFF'S SURFACE INTEGRAL

Let us assume a seismic pulse generated by a sudden application of unit pressure, such as the explosion of dynamite in a hole in the ground; in a spherical cavity of such relatively small radius that we treat the source hole as a point source under a plane boundary S. At any time t after the explosion an observer, situated at a point Q within the medium bounded by S will "see" the reflected image of rays diverging from the simple source at a point $0'$ (Figure 1); their intersection with the surface S taking the form of a closed curve. From this section of the surface plane, acting as a secondary source, rays converge at Q, which is the "eye" of the "observer."

We use rectilinear coordinates with the surface location of the source point as origin and the point Q in the xz-plane with the point 0. We take the z-axis of our configuration as positive downward, the inward normal to S.

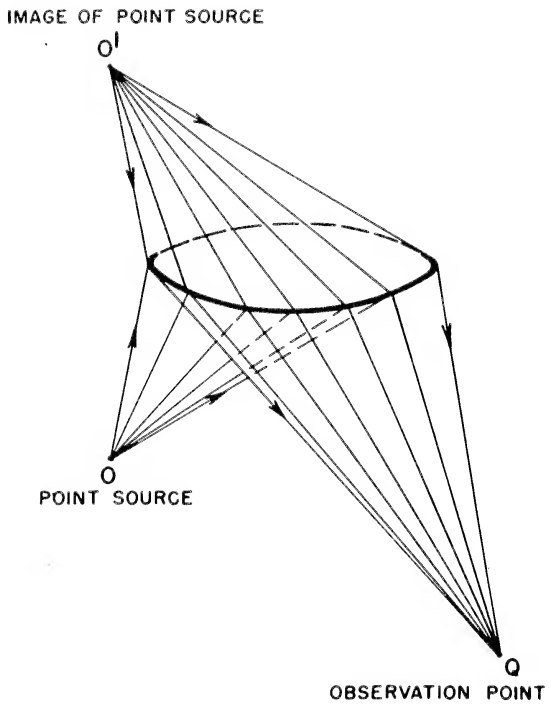


Figure 1. A sketch illustrating the point source image concept.

Kirchhoff's formula which follows (Baker and Copson, 1939) states a general theorem concerning solutions of the scalar wave equation with constant coefficients:

Let $u(x, y, z, t)$ be a solution of the equation

$$\nabla^2 u = (1/V^2)\,\delta^2 u/\delta t^2 \qquad (\nabla^2 \text{ is Laplace's operator})$$

whose partial derivatives of the first and second orders are continuous within and on a closed surface S, and let $(x_1, y_1, z_1)$ be a point within S.

Then,

$$u(x_1, y_1, z_1, t) =$$

$$(1/4\pi)\iint\{|u|\delta(1/r)/\delta n - (1/Vr)\delta r/\delta n|\delta u/\delta t| - (1/r)|\delta u/\delta n|\}dS, \qquad (1)$$

where r is the distance from $(x_1, y_1, z_1)$ to a typical point of S, $\delta/\delta n$ denotes differentiation along the inward normal to S, square brackets ($|\ |$) indicate retarded values, and V denotes wave velocity.

If, however, the point $(x_1, y_1, z_1)$ lies outside S, the value of the integral is zero.

We define the retarded value of a function u at a variable point P relative to a fixed point Q, when the distance QP is r, as

$$|u| = u(x, y, z, t-r/V). \tag{2}$$

We assume a general form of the incident dilatational wave generated by the explosion at the source 0(0, 0, d) and set the scalar potential $\Delta_i$ for the incident wave equals $f_i(V_c t - r_0)/r_0$, where $f_i$ is some dimensionless function of the argument $(V_c t - r_0)$, and $r_0$ is the distance from the point 0(0, 0, d) to a point $P(x, y, z \ll \varepsilon)$, in the direction of the propagation of the incident wave, on the surface S (Figures 2-1 and 2-2). We use the very small figure $\varepsilon$ to indicate the proximity of Kirchhoff's surface S to our xy-plane of the earth's surface. Kirchhoff's S is a mathematical surface and the integral is discontinuous at the boundary. In setting z = 0 after differentiation, we are approximating the values of the coefficients in the integral.

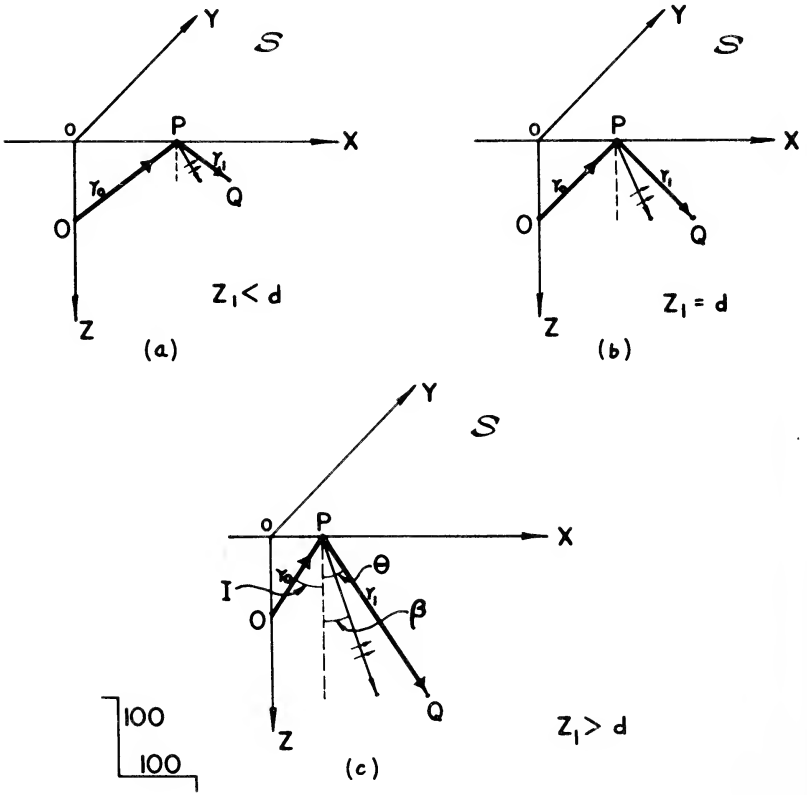


Figure 2-1. Geometry of the data used in the 3 numerical examples for the compressional wave equal-time rings.

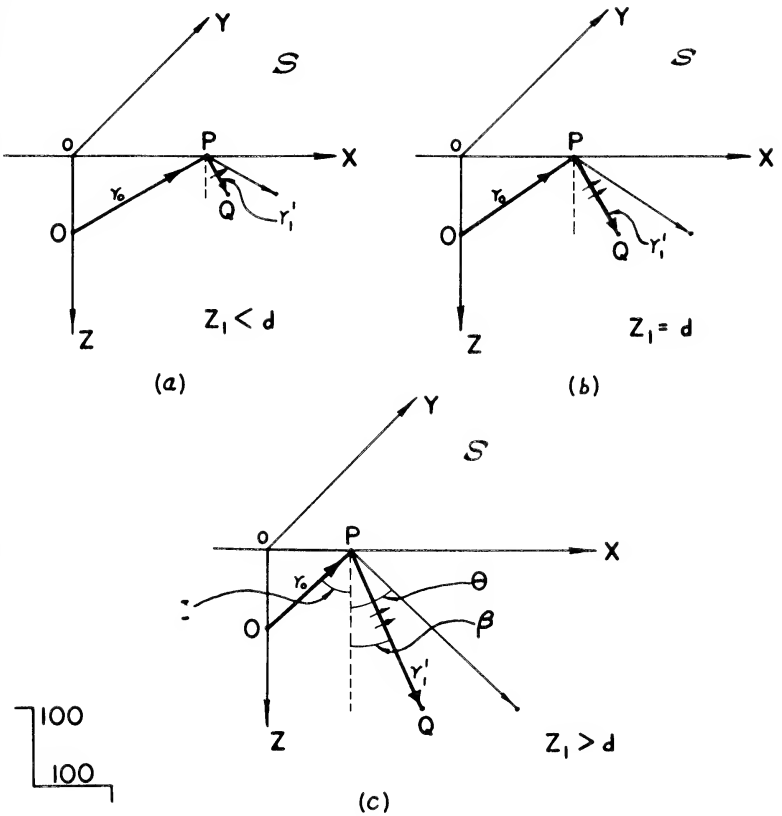


Figure 2-2. Geometry of the incident compressional wave originating at point source 0(0, 0, d) and reflected from surface S at a different point P(x, 0, 0) with the reflected shear wave arriving at point $Q(x_1, 0, z_1)$.

Let us approximate the spherical wave at $P(x, y, z \ll \varepsilon)$ by a plane wave, and in differentiating in the direction of the normal require that $r_0$ be constant for a given P, but allow both the amplitude and direction to vary for different points on S.

Now, consider the reflection of a plane compressional wave incident on a free surface which bounds an isotropic, homogeneous, perfectly elastic medium occupying a half space. We consider only the energies of the 2 independent reflected waves, the compressional and the shear. To develop the scalar potential, $\Delta_r(x_1, y_1, z_1, t)$, for the reflected compressional wave at $Q(x_1, y_1, z_1)$ we substitute $H(I)\Delta_i$ for the u of Kirchhoff's formula, z for n, $r_1$ for r, and $V_c$ for V (Figure 2-1). To develop the vector potential, $\vec{R}(x_1, y_1, z_1, t)$, for the reflected shear wave, we substitute $G(I)\Delta_i$ for u, z for n, $r_1'$ for r, and $V_s$ for V (Figure 2-2). The functions H(I) and G(I) are the compressional and shear wave reflection coefficients for incident dilatational waves, respectively, for a free surface (McLemore and Wright, 1976).

Then,

$$\Delta_r(x_1, y_1, z_1, t) \simeq (1/4\pi)\iint \{|H(I)\Delta_i| \delta(1/r_1)/\delta z - (1/V_c r_1)\delta r_1/\delta z \,|\delta(H(I)\Delta_i)/\delta t| - (1/r_1)|\delta(H(I)\Delta_i)/\delta z|\}\, dS; \quad (3)$$

and

$$\vec{R}(x_1, y_1', z_1, t) \simeq (1/4\pi)\iint \{|G(I)\Delta_i| \delta(1/r_1')/\delta z - (1/V_s r_1')\delta r_1'/\delta z \,|\delta(G(I)\Delta_i)/\delta t| - (1/r_1')|\delta(G(I)\Delta_i)/\delta z|\}\, dS. \quad (4)$$

In making the substitutions of these reflection coefficients it should be understood that Kirchhoff's formula applies so long as the path structure is large compared with the wave length.

Performing the indicated differentiation in (3) and (4), see Appendix A, and substituting our definition of retarded values in (2), we get

$$\Delta_r(x_1, y_1, z_1, t) \simeq (1/4\pi)\iint (1/r_0 r_1)\{(H(I)(\text{Cos}I/r_0 + \text{Cos}\,\theta/r_1) + (dH(I)/dI)(\text{Sin}\, I/r_0))\cdot f_i(V_c t - (r_0 + r_1)) + (H(I)(\text{Cos}\, I + \text{Cos}\,\theta)\cdot f_i'(V_c t - (r_0 + r_1))\}\, dS; \quad (5)$$

and

$$\vec{R}(x_1, y_1, z_1, t) \simeq (1/4\pi)\iint (1/r_0 r_1')\{(G(I)(\text{Cos}\, I/r_0 + \text{Cos}\,\beta/r_1') + (dG(I)/dI)(\text{Sin}\, I/r_0))\cdot f_i(V_c t - (r_0 + ar_1')) + G(I)(\text{Cos}\, I + a\text{Cos}\,\beta)\cdot f_i'(V_c t - (r_0 + ar_1'))\}dS, \quad (6)$$

where $a = V_c/V_s$, the ratio of the reflected compressional wave velocity to the reflected shear wave velocity.

From (5) we get the coefficient of the wave function $f_i(V_c t - (r_0 + r_1))$ for the reflected compressional wave as

$$FH \simeq (1/r_0 r_1)\{(H(I)(\text{Cos}\, I/r_0 + \text{Cos}\,\theta/r_1) + (dH(I)/dI)\text{Sin}\, I/r_0)\}, \quad (7)$$

and the coefficient of the wave function $f_i'(V_c t - (r_0 + r_1))$ as

$$FPH \simeq (1/r_0 r_1)\{H(I)(\text{Cos}\, I + \text{Cos}\,\theta)\}. \quad (8)$$

From (6) we get the coefficient of the wave function $f_i(V_c t - (r_0 + ar_1'))$ for the reflected shear wave as

$$FG \simeq (1/r_0 r_1')\{(G(I)(\text{Cos}\, I/r_0 + \text{Cos}\,\beta/r_1') + (dG(I)/dI)\text{Sin}\, I/r_0)\}, \quad (9)$$

and the coefficient of the wave function $f_i'(V_c t - (r_0 + ar_1'))$ as

$$FPG \simeq (1/r_0 r_1')\{G(I)(\text{Cos } I + a\text{Cos } \beta)\}. \tag{10}$$

As can be seen from the graphs of the numerical examples which follow (Figures 4-1 and 5-1), the values of the coefficients FH and FG are insignificant as compared with the values of FPH and FPG. For a first approximation we write for (5) and (6):

$$\Delta_r(x_1, y_1, z_1, t) \simeq (1/4\pi)\iint\{(1/r_0 r_1)H(I)(\text{Cos } I + \text{Cos } \theta)\cdot f_i'(V_c t - (r_0 + r_1))\}dS; \tag{5.1}$$

and

$$\vec{R}(x_1, y_1, z_1, t) \simeq (1/4\pi)\iint\{(1/r_0 r_1')G(I)(\text{Cos } I + a\text{Cos } \beta)\cdot f_i'(V_c t - (r_0 + ar_1'))\}dS; \tag{6.1}$$

where

$$H(I) = \frac{4\text{Sin}^3\beta\text{Cos }\beta\text{Cos } I - \text{Sin } I\text{Cos}^2 2\beta}{4\text{Sin}^3\beta\text{Cos }\beta\text{Cos } I + \text{Sin } I\text{Cos}^2 2\beta}, \tag{5.2}$$

and

$$G(I) = \frac{-4\text{Sin}^2\beta\text{Cos } 2\beta\text{Cos } I}{4\text{Sin}^3\beta\text{Cos }\beta\text{Cos } I + \text{Sin } I\text{Cos}^2 2\beta}, \tag{6.2}$$

$$dH(I)/dI =$$

$$\frac{(8\text{Sin}^3\beta\text{Cos } 2\beta)}{\text{Cos } \beta}\left\{\frac{2\text{Cos}^2\beta\text{Cos}^2 I(1+2\text{Sin}^2\beta) - \text{Cos } 2\beta(\text{Sin}^2\beta\text{Cos}^2 I + \text{Sin}^2 I\text{Cos}^2\beta)}{(4\text{Sin}^3\beta\text{Cos }\beta\text{Cos } I + \text{Sin } I\text{Cos}^2 2\beta)^2}\right\} \tag{5.3}$$

and

$$dG(I)/dI =$$

$$\frac{4\text{Sin}^2\beta}{\text{Sin } I\text{Cos } \beta}\left\{\frac{4\text{Sin}^3\beta\text{Cos}^3 I - \text{Sin } I\text{Cos}^2 2\beta\text{Cos } \beta(\text{Cos } 2I + 2\text{Sin}^2\beta)}{(4\text{Sin}^3\beta\text{Cos }\beta\text{Cos } I + \text{Sin } I\text{Cos}^2 2\beta)^2}\right\}; \tag{6.3}$$

where

I is the angle between $z_1$ and $r_0$,

$\theta$ is the angle between $z_1$ and $r_1$,

$\beta$ is the angle between $z_1$ and $r_1'$,

and

a is the ratio of the reflected compressional wave velocity to the reflected shear wave velocity.

## LOCI OF EQUAL-TIME RINGS ON THE SURFACE S

We are interested in the location of the secondary source points on the reflecting surface S of rays arriving at Q in phase. The phase, $(V_c t - (r_0 + r_1))$ or $(V_c t - (r_0 + ar_1'))$, of a ray arriving at Q depends on time, position of the source, position of Q, and retardation – making the problem essentially one of geometrical analysis. For any time t these two expressions are constants, and, as the velocities are constants, for any time t the expressions $(r_0 + r_1)$ and $(r_0 + ar_1')$ are other constants.

*Equal-time Rings for the Compressional Wave*

The expression $V_c t - (r_0 + r_1) = 0$ represents a family of confocal prolate spheroids, or ellipsoids of revolution, with foci at 0 and Q. The intersection of this 3-dimensional system of curves with the earth's surface – our S as the plane $z = 0$ – is a family of eccentric ellipses, symmetrical with respect to the x-axis (Figure 3). These curves define the loci on the reflecting surface S corresponding to equal-time rings for the reflected compressional waves (Rayleigh, 1877). Their eccentricity depends on the ratio of the depth of the point Q to the depth of the point 0, and on the $x_1$ coordinate.

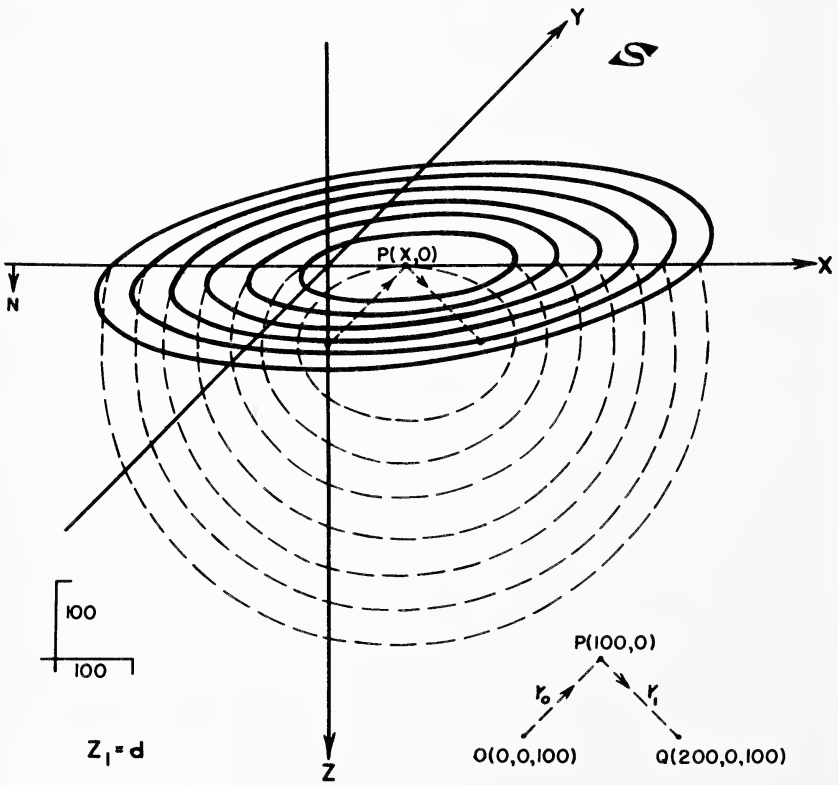


Figure 3. XZ-Cross-section of the ellipsoids with perspective view of the corresponding compressional wave equal-time rings.

The larger the ellipsoids the later the arrival. The earliest arrival corresponds to the ellipsoid just tangent to the surface. This point of tangency is the ordinary reflection point, as the tangent plane to an ellipsoid makes equal angles with the focal radii at the point of tangency, and the angle of incidence is equal to the angle of reflection of the compressional wave at this point. The rings are concentric when the foci 0 and Q are at equal distances from the reflecting surface, with center at the reflection point equal to $1/2x_1$. For $V_c t >> \overline{0Q}$, the ellipses become concentric circles, with center equal to $1/2x_1$. If the Points 0 and Q are on the same vertical, the rings are concentric circles with the reflection point and center at the origin.

If we let $(r_0 + r_1) = k$, and substitute the coordinate values of $r_0$ and $r_1$, rationalization yields (Appendix B, Figure 2-1)

$$(x - 1/2x_1 K/(k^2 - x_1^2))^2 + (k^2/(k^2 - x_1^2))y^2 = (k^2/4(k^2 - x_1^2)^2)(K^2 - 4d^2(k^2 - x_1^2)), \quad (11)$$

where

$$K = ((k^2 - x_1^2) + (d^2 - z_1^2)) \geq d^2.$$

Equation (11) defines a system of ellipses with center at

$$((1/2x_1 K)/(k^2 - x_1^2), 0, 0),$$

major semi-axis equal to

$$(k/2(k^2 - x_1^2))(K^2 - 4d^2(k^2 - x_1^2))^{1/2},$$

and minor semi-axis equal to

$$(1/2(k^2 - x_1^2)^{1/2})(K^2 - 4d^2(k^2 - x_1^2))^{1/2}.$$

The value of the parameter k for the reflection point, which we denote by $k_0$ is

$$k_0 = (x_1^2 + (z_1 + d)^2)^{1/2}, \quad (12)$$

and the reflection point is at the value of x for which

$$K^2 = 4d^2(k^2 - x_1^2),$$

or,

$$x = (d/(d + z_1))x_1. \quad (13)$$

If we let $\Delta k$ represent a constant difference between succeeding values of k, then a unit change of $\Delta k$ in k is equal to a unit change in distance corresponding to a unit change in retardation of time. For consistency in comparing potential values at various observation points, $Q(x_1, 0, z_1)$, we can set

$$\Delta k = k_0 - \overline{0Q}$$

and

$$k_n = k_0 + n\Delta k, \qquad \text{where } n = 1, 2, 3, \ldots .$$

However, as most of the energy represented by the functions occurs immediately after reflection, we can locate the maximum effect by subdividing the first interval (n = 1), making the unit change of time as small as our computation errors permit. Since $k_0$ corresponds to the time of reflection, we begin the computations with $k_0 + 1$ as representing the first ring.

*Equal-time Rings for the Shear Wave*

The expression $V_c t - (r_0 + ar_1') = 0$ represents a system of surfaces of revolution, or space quartics, with foci at the Points 0 and Q. The intersection of these 3-dimensional surfaces with the reflecting surface S is a system of 2 quadratics which do not touch, or a biquadratic, symmetrical with respect to the x-axis.

If we let $r_0 + ar_1' = k'$ and substitute the coordinate values of $r_0$ and $r_1'$ for the shear wave, rationalization yields (Appendix B, Figure 2-2)

$$x^4 + 2Dx^3 + (D^2 - E)x^2 + Fx + G + y^4 + (2x^2 + 2Dx - E)y^2 = 0, \qquad (14)$$

where

$$D = -wa^2x_1, \qquad E = wK' + w^2k'^2, \qquad F = -wK'D,$$

$$G = (w^2K'^2/4) - w^2k'^2d, \qquad w = 2/(1 - a^2),$$

$$K' = ((k'^2 - a^2x_1^2) + (d^2 - a^2z_1^2)) \geq d^2,$$

and, by definition,

$$a = V_c/V_s.$$

We solve this quartic for y in terms of given values of x. Setting the expression

$$x^4 + 2Dx^3 + (D^2 - E)x^2 + Fx + G = A,$$

and the expression

$$2x^2 + 2Dx - E = B,$$

equation (14) becomes

$$y^4 + By^2 + A = 0, \qquad (15)$$

a biquadratic system for the parameters k′ and a, containing the 2 quadratics

$$y^2 = (-B - (B^2 - 4A)^{1/2})/2$$

and

$$y^2 = (-B + (B^2 - 4A)^{1/2})/2.$$

If $B^2 - 4A \geq 0$, the roots are real and these 2 quadratics, or one of them, represent the equal time rings for the shear wave on the surface S.

In our computations we found that the values of x and y which satisfy the quadratic

$$y^2 = (-B + (B^2 - 4A)^{1/2})/2$$

for real roots do not satisfy the original equation $r_0 + ar_1' = k'$, for the values of $k'$ used. We found that the values of x and y which satisfy the quadratic

$$y^2 = (-B - (B^2 - 4A)^{1/2})/2$$

for real roots do satisfy the original equation for the values of $k'$ used. We concluded that, for our problem, the first quadratic has no physical meaning, and that the equal-time rings which we seek for the reflected shear wave are represented by the equation

$$y^2 = (-B - (B^2 - 4A)^{1/2})/2, \tag{16}$$

a family of closed curves symmetrical with respect to the x-axis, which appear on the graph as slightly distorted ellipses. When the Points 0 and Q are on the same vertical, the rings are concentric circles with the center and reflection point at the origin.

The shear rings are larger than the compressional rings for the same point $Q(x_1, 0, z_1)$ because the value $k'$ is greater than the value k for the compressional waves, which means that the shear waves arrive at Q at a later time.

The reflection point P(x, 0, 0) for the shear wave at $Q(x_1, 0, z_1)$ is

$$x = (d/(d + z_1))x_2, \tag{17}$$

where $x_2$ is a coordinate of a point $Q'(x_2, 0, z_1)$ for the compressional wave corresponding to the Point $Q(x_1, 0, z_1)$ for the reflected shear wave. We do not know the value of $x_2$ corresponding to the value $x_1$ for the shear wave. We cannot use Equation (17) to find the reflection point.

To locate the reflection point P(x, 0, 0) for the shear wave at $Q(x_1, 0, z_1)$ we use Snell's Law which states that

$$\text{Sin } \theta/V_c = \text{Sin } \beta/V_s$$

(In this case $\theta$ is the angle between the normal n and Q'P.)

At the reflection point, Sin $\theta$ = Sin I = x/r and Sin $\beta = (x_1 - x)/r_1'$ (Figure 2-2). Substituting, we get $x/ar_0 = (x_1 - x)/r_1'$, where $a = V_c/V_s$.

Rationalization (Appendix B) yields

$$x^4 - 2x_1x^3 + (C(z_1^2 - a^2d^2) + x_1^2)x^2 + 2Ca^2d^2x_1x - Ca^2d^2x_1^2 = 0, \tag{18}$$

where

$$C = 1/(1 - a^2).$$

The reflection point is that value of x, of this quartic (18), which is less than $x_1$ and greater than $(d/(d + z_1))x_1$.

The value of $k'$ at the reflection point when x has the value of (17) is

$$k'_0 = (x^2 + d^2)^{1/2} + a((x_1 - x)^2 + z_1^2)^{1/2}. \tag{19}$$

We set

$$\Delta k' = k'_0 - \overline{0Q},$$

and

$$k'_n = k'_0 + n\Delta k', \quad \text{where } n = 1, 2, 3, \ldots .$$

Using equations (7), (8), (11), (12), and (13) for the reflected compressional wave and equations (9), (10), (14), (15), (16), (17), (18), and (19) for the reflected shear wave, we wrote a general FORTRAN IV program for computation of a plane wave approximation of the velocity potentials created by the reflection of a spherical incident compressional wave on a stress free surface.

Figures 4, 4-1, 5, and 5-1 show 2 numerical examples of the reflecting surface S and the resulting values of the coefficients of the wave functions, at a given point within the medium through which the sound waves travel as time passes. The coefficients assume the greatest magnitude when the source point is on the same vertical with the observation point. When the observation point is near the reflecting surface and the origin, the compressional wave coefficients are greater than the shear wave coefficients. As the observation point is moved away from the origin and the reflecting surface, the shear wave coefficients are greater than those of the compressional wave. Most of the effect occurs immediately after reflection.

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Figure 4. (See page 73.) Equal-time rings on surface S for the 2 positions of the observation point Q(10, 0, 10) and Q(200, 0, 200), with d = 100 feet and velocity ratio $a = (3)^{1/2}$. Note the compressional wave.

Figure 4-1. (See page 74.) Compressional wave coefficients corresponding to rings of Figure 4.

Figure 5. (See page 75.) Equal-time rings on surface S for the 2 positions of observation point Q(10, 0, 10) and Q(200, 0, 200), with d = 100 feet and velocity ratio $a = (3)^{1/2}$. For the shear wave.

Figure 5-1. (See page 76.) Shear wave coefficients corresponding to rings of Figure 5.

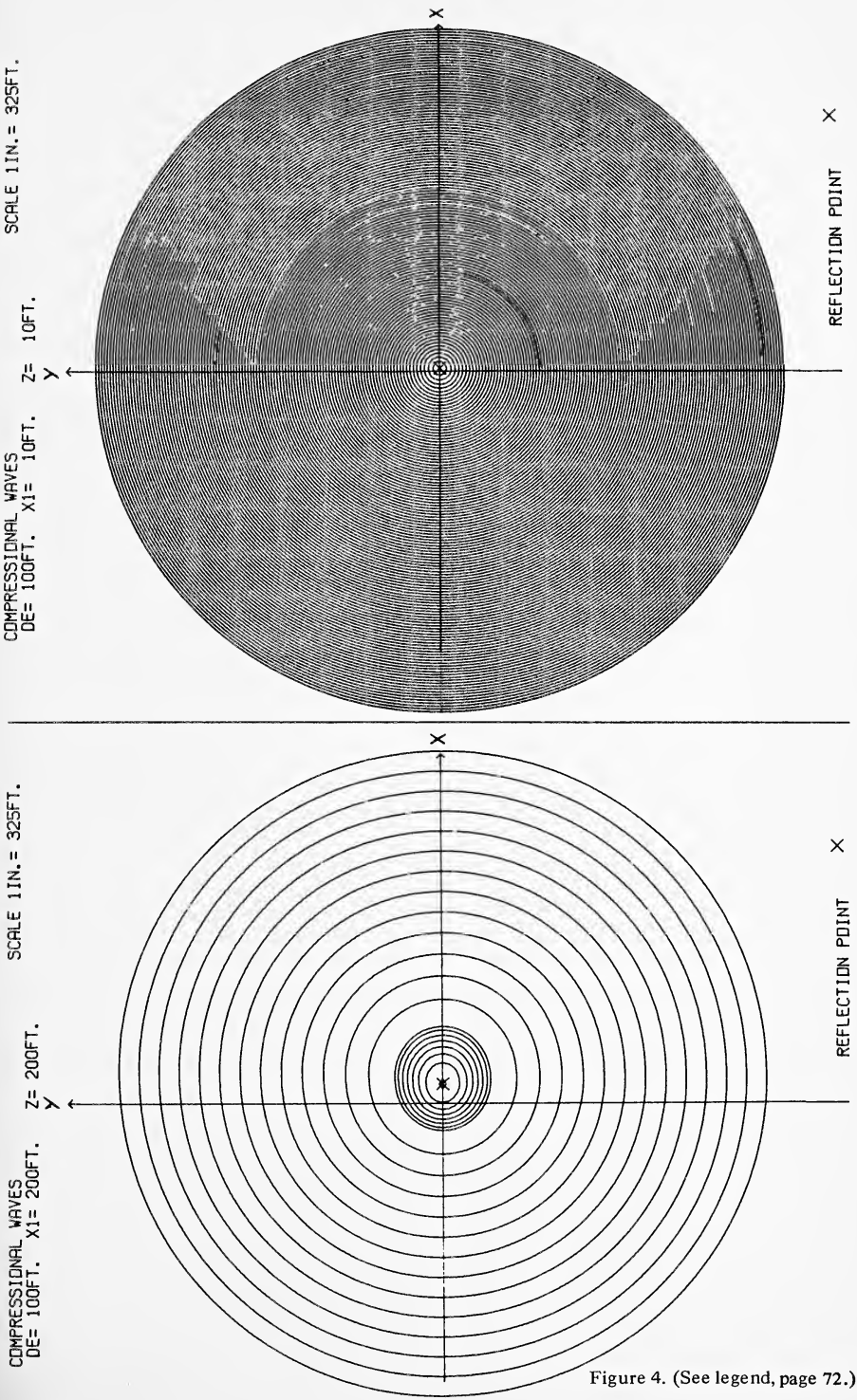
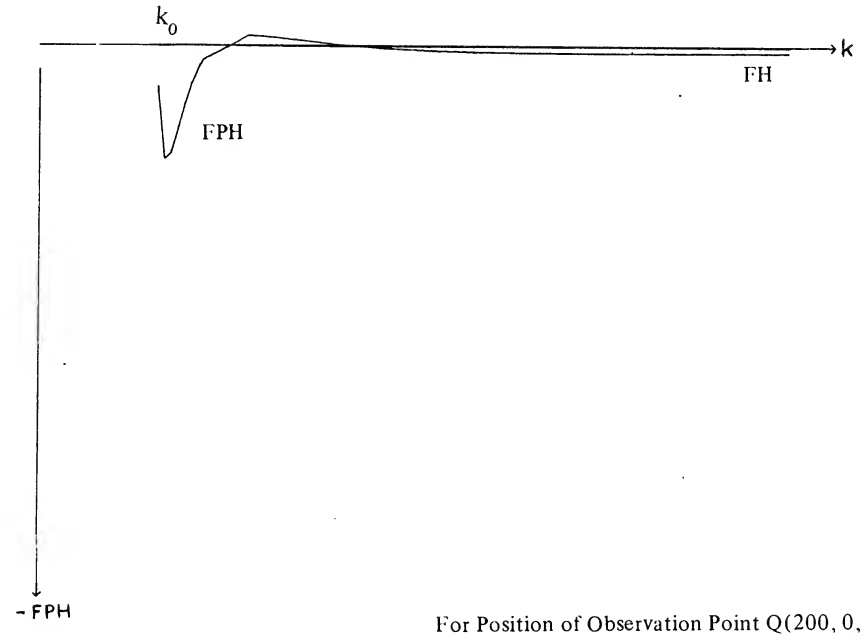
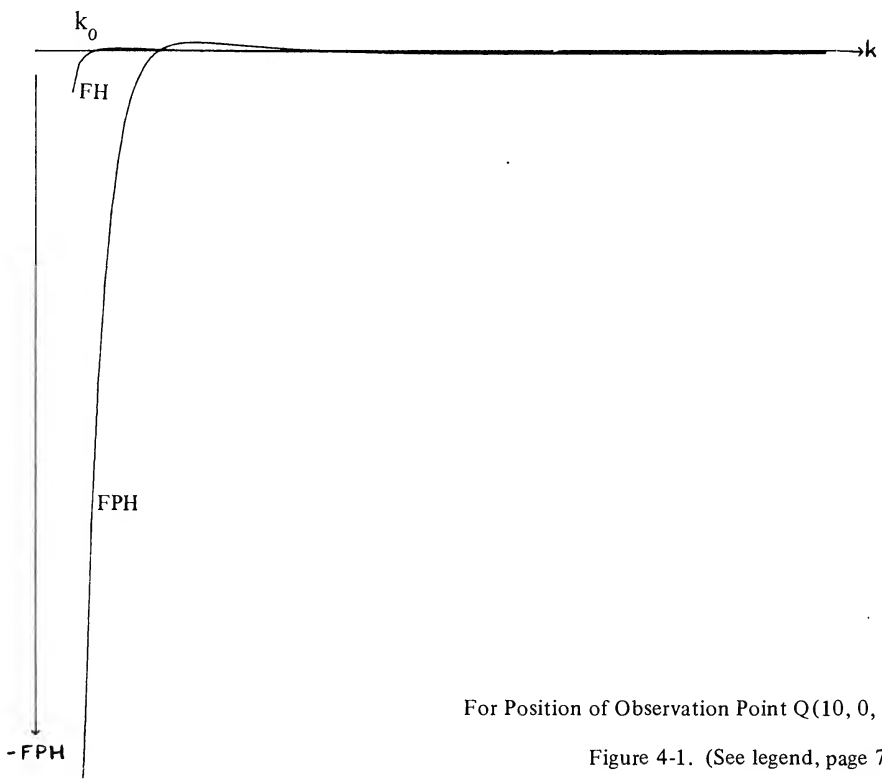



Figure 4. (See legend, page 72.)




For Position of Observation Point Q(200, 0, 200)




For Position of Observation Point Q(10, 0, 10)

Figure 4-1. (See legend, page 72.)

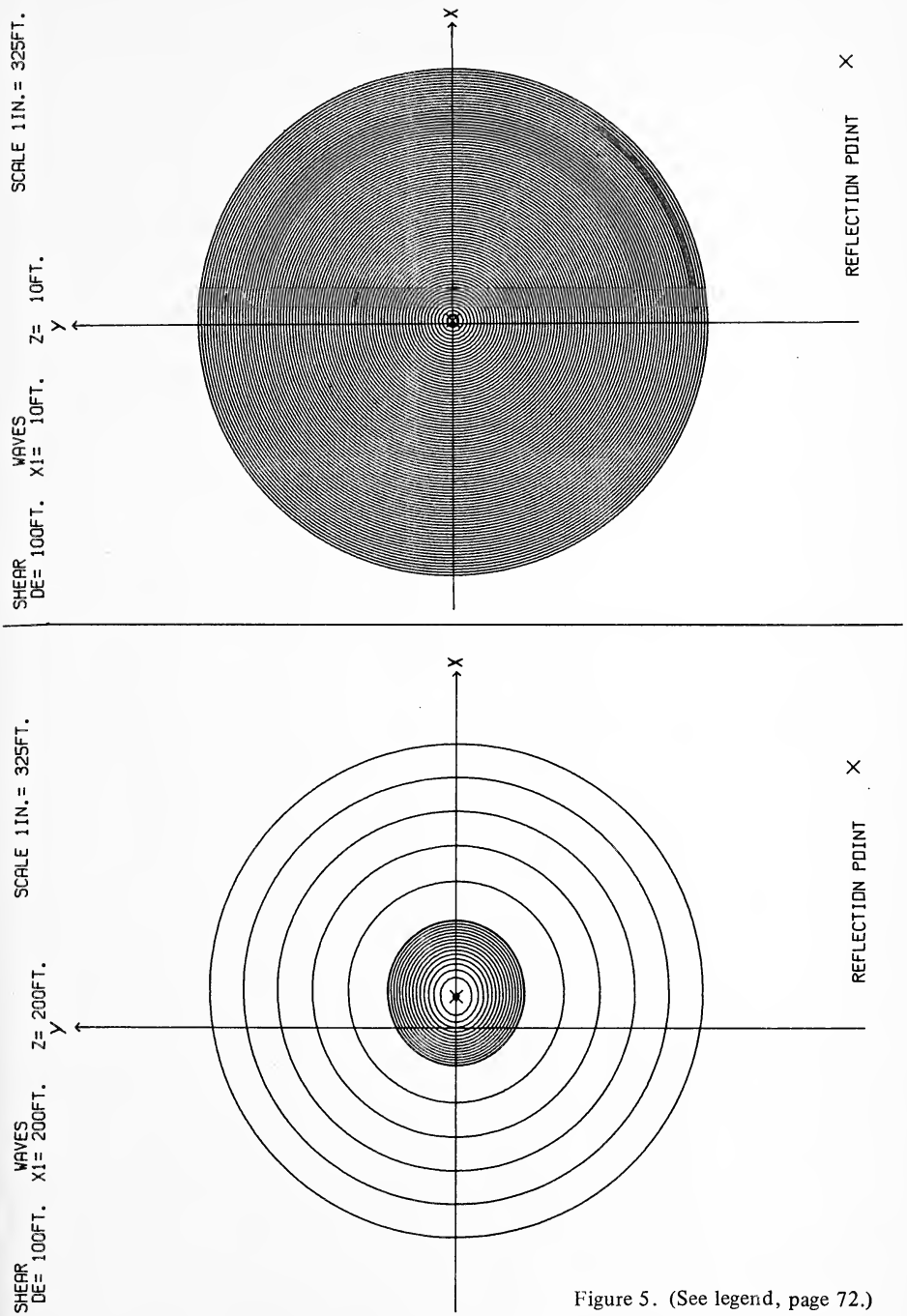
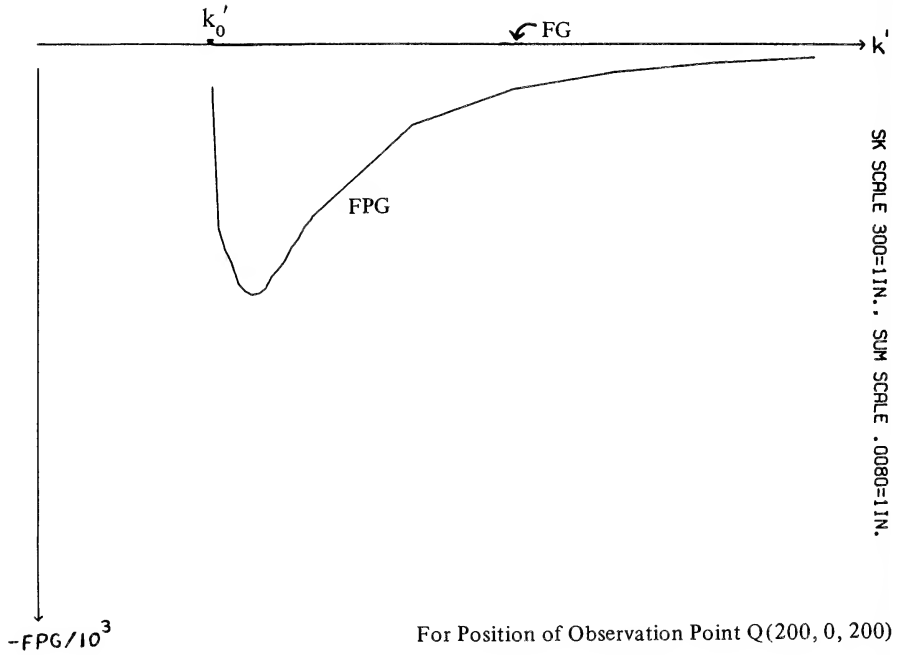
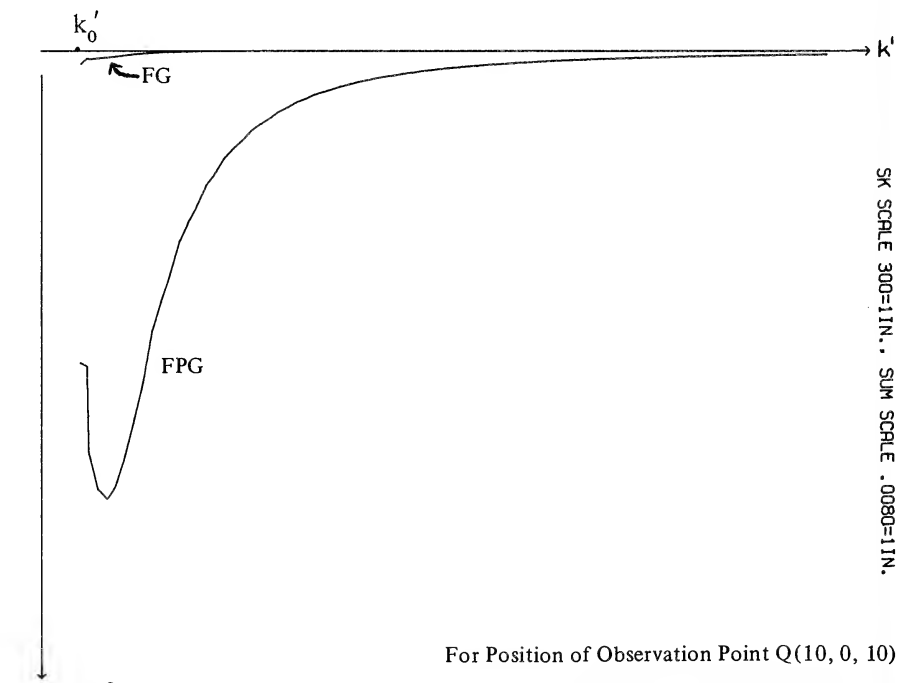



Figure 5. (See legend, page 72.)




For Position of Observation Point Q(200, 0, 200)




For Position of Observation Point Q(10, 0, 10)

Figure 5-1. (See legend, page 72.)

## APPENDIX A

The Differentiation in (3), $\Delta_r(x_1, y_1, z_1, t)$:

$$r_1 = ((x - x_1)^2 + y^2 + (z - z_1)^2)^{1/2}.$$

$$\Delta_i = f(V_c - r_0)/r_0.$$

$$\delta(1/r_1)/\delta z = (-1/r_1^2)\delta r_1/\delta z.$$

$$\begin{aligned}\delta r_1/\delta z &= \delta((x - x_1)^2 + (z - z_1)^2)^{1/2}/\delta z \\ &= 1/2((x - x_1)^2 + y^2 + (z - z_1)^2)^{-1/2} \cdot 2(z - z_1) \\ &= (z - z_1)/r_1 \\ &= -\text{Cos } \theta \qquad \text{(when } z = 0).\end{aligned}$$

$$\delta(1/r_1)/\delta z = (1/r_1^2)\text{Cos } \theta.$$

$$H(I)\Delta_i \left|\delta(1/r_1)/\delta z\right| = (1/r_0 r_1) \cdot H(I)(\text{Cos } \theta/r_1 \cdot \left|f_i(V_c t - r_0)\right|.$$

$$r_0 = (x^2 + y^2 + (z - d)^2)^{1/2}$$

$$\delta(H(I)\Delta_i)/\delta t = H(I)\delta\Delta_i/\delta t + \Delta_i \delta H(I)/\delta t.$$

$$\begin{aligned}\delta\Delta_i/\delta t &= \delta((1/r_0)f_i(V_c t - r_0))/\delta t \\ &= (1/r_0)\delta(f_i(V_c t - r_0))/\delta t \\ &= (1/r_0) \cdot V_c \cdot f_i'(V_c t - r_0),\end{aligned}$$

where $f_i'$ is the derivative of $f_i$ with respect to the argument.

$$\delta H(I)/\delta t = 0 \qquad (H(I) \text{ contains no } t).$$

$$\delta(H(I)\Delta_i)/\delta t = (1/r_0) \cdot H(I) \cdot V_c \cdot f_i'(V_c t - r_0).$$

$$-(1/V_c r_1)\delta r_1/\delta z \left|\delta(H(I)\Delta_i)/\delta t\right| = (1/r_0 r_1) \cdot H(I) \cdot \text{Cos } \theta \cdot f_i'(V_c t - r_0).$$

$$-(1/r_1)\left|\delta(H(I)\Delta_i)/\delta z\right| = -(1/r_1)\left|\Delta_i \delta H(I)/\delta z + H(I)\delta\Delta_i/\delta z\right|.$$

$$\delta(H(I))/\delta z + (dH(I)/dI/dz) = -(\text{Sin } I/r_0)dH(I)/dI.$$

$$\Delta_i \delta H(I)/\delta z = (-(\text{Sin } I/r_0)dH(I)/dI) \cdot f_i(V_c t - r_0)/r_0.$$

$$\begin{aligned}H(I)\delta\Delta_i/\delta z &= (H(I))\delta((1/r_0)f_i(V_c t - r_0)) \cdot \delta z \\ &= (H(I))f_i(V_c t - r_0)\delta(1/r_0)/\delta z + (H(I))\delta f_i(V_c t - r_0)/\delta z \cdot (1/r_0).\end{aligned}$$

$$\begin{aligned}\delta(1/r_0)/\delta z &= -(1/r_0^2)\delta r_0/\delta z \\ &= -(1/r_0^2) \cdot 1/2(x^2 + y^2 + (z - d)^2)^{-1/2} \cdot 2(z - d) \\ &= -(1/r_0^2) \cdot (z - d)/r_0 \\ &= -(1/r_0^2)\text{Cos } I \qquad \text{(when } z = 0).\end{aligned}$$

$$H(I)f_i(V_c t - r_0)\delta(1/r_0)/\delta z = -(1/r_0) \cdot H(I) \cdot (\text{Cos } I/r_0) \cdot f_i(V_c t - r_0).$$

$$\begin{aligned}\delta f_i(V_c t - r_0)/\delta z &= \delta(V_c t - r_0)/\delta z \cdot f_i'(V_c t - r_0) \\ &= -\text{Cos } I \cdot f_i'(V_c t - r_0).\end{aligned}$$

Appendix A (Continued)

$$H(I)\delta f_i(V_c t - r_0)/\delta z \cdot (1/r_0) = -(1/r_0)H(I) \cdot \text{Cos } I \cdot f_i'(V_c t - r_0).$$

$$H(I)\delta\Delta_i/\delta z = -(1/r_0) \cdot H(I) \cdot (\text{Cos } I/r_0) \cdot f_i(V_c t - r_0) - (1/r_0) \cdot H(I) \cdot \text{Cos } I \cdot f_i'(V_c t - r_0).$$

$$-(1/r_1)\left|\delta(H(I)\Delta_i)/\delta z\right| = (1/r_0 r_1)((dH(I)/dI)(\text{Sin } I/r_0) \cdot \left|f_i(V_c t - r_0)\right|$$
$$+ (1/r_0 r_1)H(I)(\text{Cos } I/r_0) \cdot \left|f_i(V_c t - r_0)\right|$$
$$+ (1/r_0 r_1)H(I)\text{Cos } I \cdot \left|f_i'(V_c t - r_0)\right|.$$

Substituting these values for the three terms in (3) and substituting our value of retarded time (2) where $t = t - r_1/V_c$, we get Equation (5), page 66;

$$\left|f_i(V_c t - r_0)\right| = f_i(V_c t - (r_0 + r_1)),$$

where

$$\left|f_i'(V_c t - r_0)\right| = f_i'(V_c - (r_0 + r_1)).$$

The Differentiation in (4) $\vec{R}(x_1, y_1, z_1, t)$:

$$r_1' = (x - x_1)^2 + y^2 + (z - z_1)^2)^{1/2}.$$

$$\delta(1/r_1')/\delta z = (-1/r_1'^2)\delta r_1'/\delta z.$$

$$\delta r_1'/\delta z = (z - z_1)/r_1'$$
$$= -\text{Cos } \beta \qquad (\text{when } z = 0).$$

$$\delta(1/r_1')/\delta z = (1/r_1'^2)\text{Cos } \beta.$$

$$\left|G(I)\Delta_i\right|\left|\delta(1/r_1')/\delta z\right| = (1/r_0 r_1') \cdot G(I)(\text{Cos } \beta/r_1') \cdot \left|f_i(V_c t - r_0)\right|.$$

$$\delta(G(I)\Delta_i)/\delta t = G(I)\delta\Delta_i/\delta t + \Delta_i \delta G(I)/\delta t.$$

$$\delta\Delta_i/\delta t = (1/r_0) \cdot V_c \cdot f_i'(V_c t - r_0).$$

$$\delta G(I)/\delta t = 0 \qquad (G(I) \text{ contains no } t).$$

$$\delta(G(I)\Delta_i)/\delta t = (1/r_0) \cdot G(I) \cdot V_c \cdot f_i'(V_c t - r_0).$$

$$-(1/V_s r_1')\delta r_1'/\delta z\left|\delta(G(I)\Delta_i)/\delta t\right| = (1/r_0 r_1') \cdot G(I) \cdot (V_c/V_s) \cdot \text{Cos } \beta \cdot \left|f_i'(V_c t - r_0)\right|.$$

$$-(1/r_1')\left|\delta(G(I)\Delta_i)/\delta z\right| = -(1/r_1')\left|\Delta_i \delta G(I)/\delta z + G(I)\delta\Delta_i/\delta z\right|.$$

$$\delta(G(I))/\delta z = (dG(I)/dI)(dI/dz)$$
$$= -(\text{Sin } I/r_0)dG(I)/dI.$$

$$\Delta_i \delta G(I)/\delta z = (-(\text{Sin } I/r_0)dG(I)/dI) \cdot f_i(V_c t - r_0)/r_0.$$

$$G(I)\delta\Delta_i/\delta z = (G(I))\delta((1/r_0)f_i(V_c t - r_0))/\delta z$$
$$= (G(I))f_i(V_c t - r_0)\delta(1/r_0)/\delta z + (G(I))\delta f_i(V_c t - r_0)/\delta z \cdot (1/r_0).$$

$$\delta(1/r_0)/\delta z = -(1/r_0^2)\text{Cos } I \qquad (\text{when } z = 0).$$

$$G(I)f_i(V_c t - r_0)\delta(1/r_0)/\delta z = -(1/r_0) \cdot G(I) \cdot (\text{Cos } I/r_0) \cdot f_i(V_c t - r_0).$$

$$\delta f_i(V_c t - r_0)/\delta z = -\text{Cos } I \cdot f_i'(V_c t - r_0).$$

Appendix A (Continued)

$$G(I)\delta f_i(V_c t - r_0)/\delta z \cdot (1/r_0) = -(1/r_0)G(I) \cdot \text{Cos } I \cdot f_i'(V_c t - r_0).$$

$$G(I)\delta\Delta_i/\delta z = -(1/r_0) \cdot G(I) \cdot (\text{Cos } I/r_0) \cdot f_i(V_c t - r_0) - (1/r_0) \cdot G(I) \cdot \text{Cos } I \cdot f_i'(V_c t - r_0).$$

$$-(1/r_1') \left| \delta(G(I)\Delta_i)/\delta z \right| = (1/r_0 r_1')((dG(I)/dI)(\text{Sin } I/r_0) \cdot \left| f_i(V_c t - r_0) \right|$$
$$+ (1/r_0 r_1')G(I)(\text{Cos } I/r_0) \cdot \left| f_i(V_c t - r_0) \right|$$
$$+ (1/r_0 r_1')G(I)\text{Cos } I \cdot \left| f_i'(V_c t - r_0) \right|.$$

Substituting these values for the 3 terms in (4) and substituting our value of retarded time (2) where $t = (t - r_1'/V_s)$, we get Equation (6), page 66;

$$\left| f_i(V_c t - r_0) \right| = f_i(V_c t - (r_0 + a r_1')),$$

where

$$\left| f_i'(V_c t - r_0) \right| = f_i'(V_c t - (r_0 + a r_1')),$$

and

$$a = V_c/V_s.$$

## APPENDIX B

## THE RATIONALIZATION

*Compressional Wave*

$$r_0 = (x^2 + y^2 + d^2)^{1/2}$$

$$r_1 = ((x - x_1)^2 + y^2 + z_1^2)^{1/2}$$

$$r_0 + r_1 = k$$

$$r_1 = k - r_0$$

$$r_1^2 = k^2 - 2kr_0 + r_0^2$$

$$x^2 - 2x_1x + x_1^2 + y^2 + z_1^2 - x^2 - y^2 - d^2 - k^2 = -2kr_0$$

$$-2x_1x + x_1^2 + z_1^2 - d^2 - k^2 = -2kr_0$$

Set $K = ((k^2 - x_1^2) + (d^2 - z_1^2))$

$$2x_1x + K = 2k(x^2 + y^2 + d^2)^{1/2}$$

$$4x_1^2x^2 + 4Kx_1x + K^2 = 4k^2x^2 + 4k^2y^2 + 4k^2d^2$$

$$(k^2 - x_1^2)x^2 - Kx_1x + k^2y^2 = K^2/4 - k^2d^2$$

$$x^2 - (Kx_1/(k^2 - x^2))x + (k^2/(k^2 - x^2))y^2 = K^2/4(k^2 - x_1^2) - k^2d^2/(k^2 - x_1^2)$$

$$x^2 - 2(Kx_1/2(k^2 - x_1^2))x + K^2x_1^2/4(k^2 - x_1^2)^2 + (k^2/(k^2 - x_1^2))y^2$$
$$= K^2/4(k^2 - x_1^2) - k^2d^2/(k^2 - x_1^2) + K^2x_1^2/4(k^2 - x_1^2)^2.$$

$$(x - Kx_1/2(k^2 - x_1^2))^2 + (k^2/(k^2 - x_1^2))y^2$$
$$= (K^2 - 4k^2d^2)/4(k^2 - x_1^2) + K^2x_1^2/4(k^2 - x_1^2)^2.$$

$$(x - Kx_1/2(k^2 - x_1^2))^2 + (k^2/(k^2 - x_1^2))y^2$$
$$= (k^2/4(k^2 - x_1^2)^2)(K^2 - 4d^2(k^2 - x_1^2)). \qquad (11)$$

*Shear Wave*

$$r_0 = (x^2 + y^2 + d^2)^{1/2}$$

$$r_1' = ((x - x_1)^2 + y^2 + z_1^2)^{1/2}$$

$$r_0 + ar_1' = k'$$

$$ar_1' = k' - r_0$$

$$a^2r_1^2 = k'^2 - 2k'r_0 + r_0^2$$

$$a^2x^2 - 2a^2x_1x + a^2x_1^2 + a^2y^2 + a^2z_1^2 - k'^2 - x^2 - y^2 - d^2 = -2k'r_0.$$

$$(a^2 - 1)x^2 + (a^2 - 1)y^2 - 2a^2x_1x + a^2x_1^2 + a^2z_1^2 - k'^2 - d^2 = -2k'r_0.$$

Set $K' = ((k'^2 - a^2x_1^2) + (d^2 - a^2z_1^2))$

$$(a^2 - 1)x^2 + (a^2 - 1)y^2 - 2a^2x_1x - K' = -2k'(x^2 + y^2 + d^2)^{1/2}.$$

Set $w = 2/(a^2 - 1)$

$$(x^2 + y^2) - wa^2x_1x - wK'/2 = -wk'(x^2 + y^2 + d^2)^{1/2}$$

Appendix B (Continued)

$$(x^2 + y^2)^2 + 2(x^2 + y^2)(-wa^2x_1x - wK'/2) + (-wa^2x_1x - wK'/2)^2$$
$$= w^2k'^2x^2 + w^2k'^2y^2 + w^2k'^2d^2.$$
$$x^4 - 2wa^2x_1x^3 + (w^2a^4x_1^2 - wK' - w^2k'^2)x^2 + w^2a^2x_1K'x + (w^2K'^2/4) - w^2k'^2d^2$$
$$+ (2x^2 - 2wa^2x_1x - wK' - w^2k'^2)y^2 + y^4 = 0.$$

Set $D = -wa^2x_1$,

$E = wK' + w^2k'^2$,

$F = -wK'D$,

$G = (w^2K'^2/4) - w^2k'^2d^2$.

Then

$$x^4 + 2Dx^3 + (D^2 - E)x^2 + Fx + G + (2x^2 + 2Dx - E)y^2 + y^4 = 0. \qquad (14)$$

*The Reflection Point for the Shear Wave*

$$r_0 = (x^2 + d^2)^{1/2}$$
$$r_1' = ((x - x_1)^2 + z_1^2)^{1/2}$$
$$\text{Sin } \beta = (1/a)\text{Sin } \theta$$
$$= (1/a)\text{Sin I}$$
$$= (x_1 - x)/r_1'$$
$$x/ar_0 = (x_1 - x)/r_1'$$
$$x^2/a^2(x^2 + d^2) = (x_1^2 - 2x_1x + x^2)/(x^2 - 2x_1x + x_1^2 + z_1^2)$$
$$(1 - a^2)x^4 - 2x_1(1 - a^2)x^3 + (z_1^2 - a^2d^2 + (1 - a^2)x_1^2)x^2 + 2a^2d^2x_1x - a^2d^2x_1^2 = 0.$$

Set $C = 1/(1 - a^2)$

$$x^4 - 2x_1x^3 + (C(z_1^2 - a^2d^2) + x_1^2)x^2 + 2Ca^2d^2x_1x - Ca^2d^2x_1^2 = 0. \qquad (18)$$

## NOTATION

| | |
|---|---|
| S | is a plane surface taken as the xy-plane. For the approximation we regard the surface S as coinciding with the earth's surface. |
| $0(0, 0, d)$ | is the source point of the explosion or disturbance and d is depth of source. The dimensions of the source are small compared with other distances involved. |
| $Q(x_1, 0, z_1)$ | is an observation point within the medium bounded by S. |
| $P(x, y, 0)$ | is any point on the surface S. |
| $r_0$ | is the distance OP. |
| $r_1$ | is the distance QP for the reflected compressional wave. |
| $r_1'$ | is the distance QP for the reflected shear wave. |
| n | is the normal into the medium. |
| I | is the angle between n and $r_0$. |
| $\theta$ | is the angle between n and $r_1$. |
| $\beta$ | is the angle between n and $r_1'$. |
| $V_c$ | is the compressional wave velocity in ft/sec. |
| $V_s$ | is the shear wave velocity in ft/sec. |
| $\sigma$ | is Poisson's ratio. |
| a | is the ratio $V_c/V_s$. |
| H(I) | is the reflection coefficient of the compressional wave at the free surface, and is a function of $V_c$, $V_s$, and I. |
| G(I) | is the reflection coefficient of the shear wave at the free surface, and is a function of $V_c$, $V_s$, and I. |
| $\Delta_i$ | is the scalar potential for the incident wave. |
| $\Delta_r$ | is the scalar potential for the reflected compressional wave. |
| $\vec{R}$ | is the vector potential for the reflected shear wave. |
| $f_i$ | is some dimensionless function of the given argument. |
| $f_i'$ | is the derivative of $f_i$ with respect to the given argument. |
| FH | is the coefficient of the wave function $f_i(V_c t - (r_0 + r_1))$. |
| FPH | is the coefficient of the wave function $f_i'(V_c t - (r_0 + r_1))$. |
| FG | is the coefficient of the wave function $f_i(V_c t - (r_0 + ar_1'))$. |
| FPG | is the coefficient of the wave function $f_i'(V_c t - (r_0 + ar_1'))$. |
| FH2 | is the value of FH for a given time. |
| FPH2 | is the value of FPH for a given time. |
| FG2 | is the value of FG for a given time. |
| FPG2 | is the value of FPG for a given time. |

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# THE EFFECT OF LYSOSOMAL ENZYMES ON CHLOROPLASTS *IN VITRO*

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ABSTRACT

Isolated chloroplasts from spinach leaves were incubated with rat liver cells in monolayer culture. The chloroplasts were phagocytized by the cells and remained visibly intact for a period of 3 hrs. The chloroplasts were incubated with the lysosomal extract from the liver cells for 15 min. There was no evidence of a release of chlorophyll or protein from the chloroplasts.

## INTRODUCTION

There are numerous examples of chloroplasts existing in an endosymbiotic relationship within the cytoplasm of certain animal cells (Taylor, 1968; Trench and Smith, 1970; Trench, *et al.,* 1972). Although these animal cells contain a variety of lysosomal enzymes capable of degrading the major categories of biologically important molecules, the chloroplasts do not appear to be digested by these enzymes. Chloroplasts phagocytized by fibroblasts in culture have retained their ultrastructural integrity for 5 cell generations (Nass, 1969). When introduced into the white of hens' eggs, isolated chloroplasts have undergone repeated division and maintained their metabolic activity as indicated by $CO_2$ fixation and Hill activity (Giles, 1972).

These observations of the cell's inability to dispose of internalized chloroplasts have prompted this further examination of the effect of lysosomal enzymes on chloroplasts.

## MATERIALS AND METHODS

After it was confirmed in our laboratory that chloroplasts were phagocytized but not degraded by rat liver cells *in vitro,* we investigated the effect of lysosomal enzymes on intact chloroplasts. Isolated chloroplasts were incubated with lysosomal enzyme extract and chloroplast degradation determined by microscopic examination and by quantitation of free chlorophyll and soluble protein.

### *Phagocytosis of Chloroplasts by Liver Cells*

Liver cells were isolated by a modification of the Dulbecco and Vogt method (Dulbecco and Vogt, 1954), suspended in TC-199 (Difco) supplemented with 20%

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calf serum, and seeded in Leighton tubes at a density of 3 - 4 x $10^5$ cells/$cm^2$. Chloroplasts were diluted with the same medium and added to the monolayer in a ratio of approximately 5:1. After 1 hr of incubation in the dark the glass coverslip was removed from the Leighton tube, inverted onto a glass slide and the cells examined by phase microscopy at 37 C (Reichert Zetopan, 1250x).

Chloroplasts were isolated from fresh spinach (Nass, 1969), and the resulting chloroplast pellet was resuspended in a modified Honda medium (Honda, *et al.*, 1966; Nass, 1969). Each preparation was examined by phase microscopy to determine the quality of the chloroplasts then stored at 4 C for no longer than 48 hrs prior to use.

*Isolation of Lysosmal Enzymes*

Three male Sprague Dawley rats (200-250 gm) were sacrificed by decapitation after fasting for 22-24 hrs. The livers were excised, weighed and minced. Five volumes of 0.25 M sucrose chilled to 4 C were added per gram of original liver tissue (5 ml/gm). The liver was then homogenized using 1 stroke of a motor driven (1000 RPM) Potter-Elvehjem homogenizer. The liver homogenate was centrifuged using a differential centrifugation procedure as described by DeDuve, *et al.* (1955). The resulting lysosomal pellet was made into 0.2% Triton X-100, refrigerated 15 min and assayed for β-glucouronidase.

*Enzyme Assay*

β-Glucouronidase was selected as the marker enzyme (Stahl and Touster, 1971) in the lysosomal extract and assayed as follows. To 0.25 ml 0.2 M sodium phenolphthalein glucouronide and 0.025 ml lysosomal extract previously made 0.2% in Triton X-100, plus sufficient water to make the total volume of the mixture to 1.0 ml. The mixture was incubated at 37 C for 15 min. The reaction was stopped with 3 ml stopping reagent (consisting of 0.13 M glycine, 0.67 M NaCl and 0.083 M $Na_2CO_3$ per liter after adjusting the pH to 10.7 with 1 N NaOH) and absorbance determined at 552 nm. The absorbance values indicated an average release of 0.013 $\mu$ moles of phenophthalein which is equivalent to 0.05 enzyme unit activity.

*Chlorophyll and Protein Determination*

To each of three 0.25 ml aliquots of chloroplast suspension the following were added: 0.25 ml sodium acetate buffer, lysosomal enzyme fraction (0.025, 0.05, and 0.1 ml), and sufficient water to make the entire incubation mixture total 1 ml. The mixtures were incubated for 15 min at 37 C with constant gentle mixing. Enzyme activity was stopped by adding 3 ml stopping reagent (described above), and the incubation mixtures were then centrifuged at 2000 x g for 7 min to remove chloroplasts and debris. The absorbance of an aliquot of the supernatant in acetone was measured at 663 and 664 nm to determine the amounts of chlorophylls a and b, and total chlorophyll in mg/g of tissue was calculated according to Arnon (1949) and Koski (1950). The values obtained were then converted into % of the total chlorophyll extracted from a sample of 0.25 ml chloroplast suspension made into 10 ml in acetone.

A modification of the Lowry method was used to determine the total protein in each sample (Campbell and Sargent, 1967; Lowry, *et al.*, 1951). The values obtained were converted into % of the amount of protein in 0.1 ml enzyme preparation.

## RESULTS AND DISCUSSION

Microscopic examination of the rat liver monolayer after 1 hr of incubation demonstrated that although the cells did phagocytize between 5 and 6 chloroplasts/cell, continuous observations for 3 hrs showed no evidence of chloroplast degradation occurring within the cytoplasm of these cells.

The amount of chlorophyll present in the supernatant of chloroplast suspensions varied with the preparation; therefore, chlorophyll values were expressed as a % of the total chlorophyll extracted from the chloroplast preparation. The mean values of free chlorophyll in the supernatants of the chloroplast control and the chloroplast-enzyme mixtures ranged from 5% to 7% (Figure 1). As a result of statistical analyses it could be inferred that the amount of chlorophyll leached from the chloroplasts into the supernatant was not influenced by lysosomal enzyme activity.

The amount of soluble protein released from the chloroplasts did not change significantly (at 0.05 probability) after incubation with the enzyme extract. It is seen in Figure 2 that the curve representing chloroplast-enzyme and the curve representing enzyme alone are parallel. The difference between the 2 curves represents the amount of soluble protein in the supernatant of 0.25 ml chloroplast suspension. As the aliquots of enzyme protein increased from 0.025 to 0.1 ml, there was a proportionate increase in the total protein of the chloroplast-enzyme supernatant. The loss of protein from chloroplasts has been previously reported by Kahn and Wettstein (1961) who found that chloroplasts isolated in an aqueous medium such as Honda medium lose much of the stromal protein within 30 min after isolation even though they appear to be structurally intact.

Earlier unpublished studies in our laboratory using neutral red as a lysosomal marker showed that human neutrophils readily phagocytize chloroplasts, but that the lysosomes do not coalesce with internalized chloroplasts. These observations of chloroplast-lysosome interaction support the work reported by other investigations (Giles, 1972; Nass, 1969; Taylor, 1968; Trench and Smith, 1970; Trench, *et al.*, 1972).

From this study there is evidence that neither chlorophyll nor protein are released from chloroplasts which have been incubated with lysosomal enzyme extract. The lysosomal enzymes are unable to degrade the chloroplast membrane under the conditions specified. Of course the possibility cannot be ruled out that the chloroplasts released an inhibitor which deactivated the lysosomal extract. These results are in agreement with the possible symbiotic origin of the chloroplast-bearing eukaryotic cells.

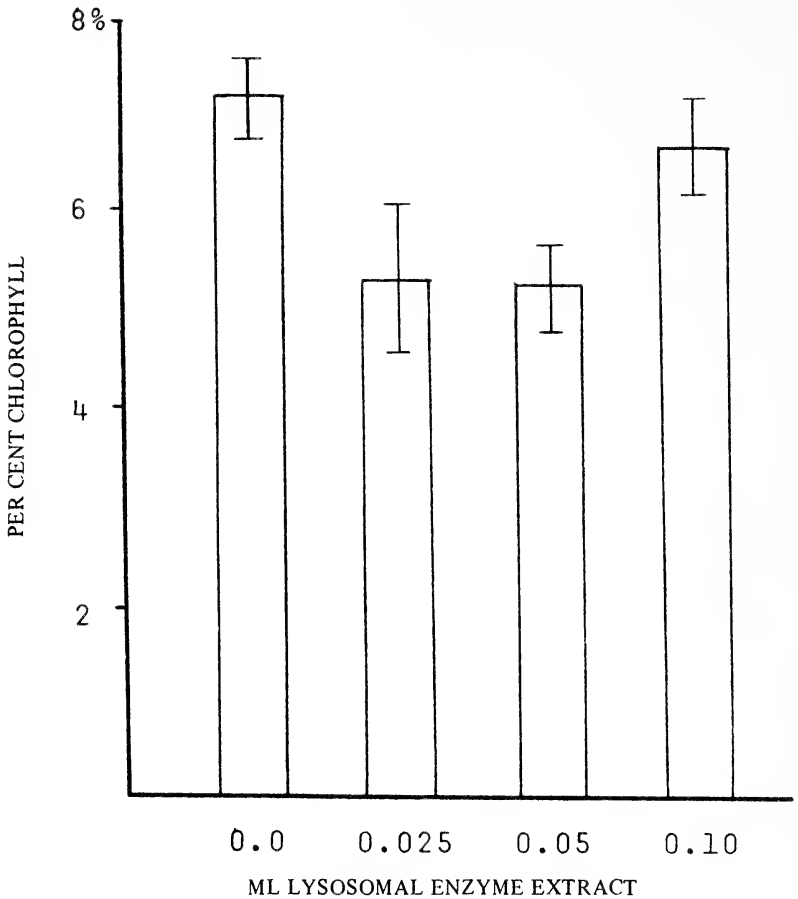


Figure 1. Chlorophyll present in supernatant of chloroplast-enzyme incubation mixtures. All samples contain 0.25 ml chloroplast suspension. Mean chlorophyll values (expressed as % of total chlorophyll extracted) with their standard error of the mean represent 2 experiments with 3 replicates each. Total chlorophyll in chloroplast suspension was assayed as 650 $\mu$g/ml.

SUMMARY

Rat liver cells in monolayer culture phagocytized isolated spinach chloroplasts; however, these internalized organelles were not visibly degraded after 3 hrs of continuous observation. Chloroplasts were incubated with lysosomal enzyme extract prepared by homogenization and differential centrifugation of rat liver, and the supernatants of chloroplast-enzyme incubation mixtures were analyzed to determine chloroplast degradation. The amount of free chlorophyll and soluble protein present in the supernatant of chlorophyll-enzyme incubation mixtures were not influenced by lysosomal enzyme activity.

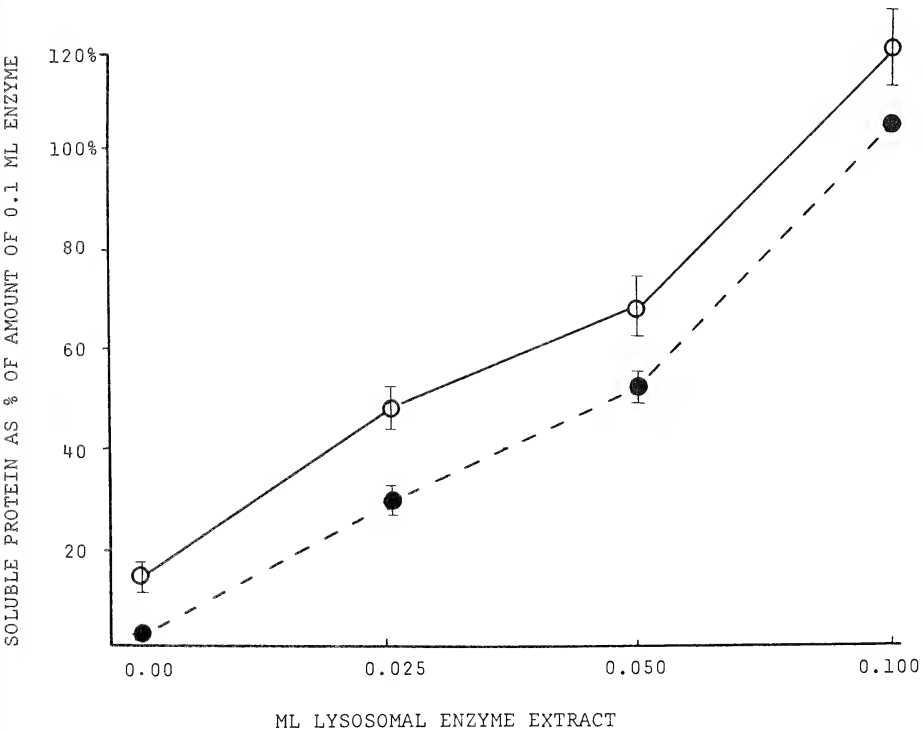


Figure 2. Amount of soluble protein from 0.25 ml chloroplast suspension incubated with lysosomal enzyme, ○——○, and soluble protein in enzyme preparation alone, ●----●, expressed as % of amount in 0.1 ml. Each value with its standard error of the mean represents 2 experiments with 3 replicates each.

ACKNOWLEDGEMENTS

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# A SIMPLE, INEXPENSIVE FREEZE-ETCH DEVICE FOR USE IN ELECTRON MICROSCOPE LABORATORIES

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ABSTRACT

A simple and inexpensive freeze-etch device for use in procedural electron microscopy courses and laboratories is described. Frozen cells are cleaved beneath the surface of liquid nitrogen, and the cleaved cell surface etched by a method which allows differential, although unregulated, heating of the holding block and the colder lid, to which ice sublimes.

## INTRODUCTION

A replica of the frozen-etched surface of biological material may be prepared by: i) rapidly freezing; ii) cleaving; iii) etching; and iv) replicating the specimen. Adhering cellular material is removed and the replica observed directly with the electron microscope. The technique of freeze-etching has been reviewed by Kohler (1968) and Bullivant (1973) who cite the advantages of freeze-etching as: i) fixation, dehydration, and embedding are not necessary; ii) cells differ from the living state only in that they are frozen, and iii) fracture proceeds along a structurally weak path, following the plasma membrane. Although previous attempts had been made to freeze-etch biological material (Steere, 1957; Haggis, 1961), Moor, *et al.* (1961), were the first to obtain consistent results with a cryoultramicrotome freeze-etch device (Balzers' apparatus). Subsequently, other workers (Koehler, 1966; Steere, 1966) developed freeze-etch devices similar to the Balzers' apparatus which allow cleaving and etching of the specimen to be performed in an evacuated chamber. In contrast to the cryoultramicrotome freeze-etch devices of Moor, *et al.* (1961), simple freeze-cleave devices have been developed (Bullivant and Ames, 1966; Winklemann and Meyer, 1968). Types of each of the simple freeze-cleave devices are commercially available, and are moderately expensive ($2,000 or more), although considerably less expensive than the Balzers' freeze-etch device. A simple,

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inexpensive freeze-etch apparatus which lacks precise temperature controls can be constructed for use in electron microscope laboratories equipped with a rotary-stage vacuum evaporator.

## MATERIALS AND METHODS

The principle components of the freeze-etch device, illustrated in Figure 1, include an insulating cup (IC), holding block (HB), and lid (L), machined from brass. The insulating cup (IC) is 3.2 cm high, 4.7 cm in diameter, with a 0.3 cm cone centered in the bottom to support the holding block. The holding block (HB), 2.6 cm high and 3.8 cm in diameter, has a small opening (arrow) in the center of

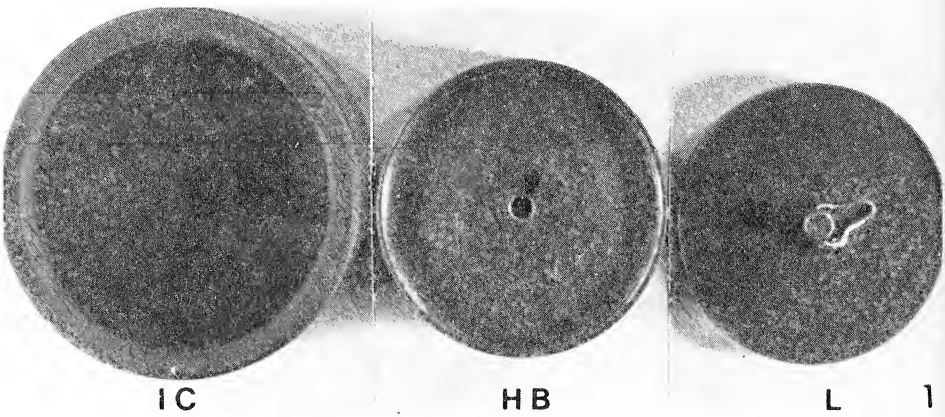


Figure 1. Photograph of specimen-holding device. Cells are placed in the hole (arrows) in the center of the holding block (HB), cleaved, covered with the lid (L), and placed in the insulating cup (IC) containing liquid nitrogen.

the block surface 0.25 cm in diameter and 0.6 cm deep. This opening holds the cells during freezing, cleavage, and replication. Damage and contamination of the cleaved cell surface is reduced by the cover (L) which is flanged to fit closely to the top of the holding block (HB). Other parts of the device, depicted on the stage of the vacuum evaporator, are an insulating platform (IP), an arm-guide (A-G) and a threaded shaft (TS) on which the arm, attached to the lid (L) by a hook (H), is raised (Figure 2).

Bacteria were prepared for freeze-etching by removing them from the surface of a solid medium and placing them in the opening in the holding block. In some cases, cells were pretreated with 28% glycerol for 30 min before freezing. The holding block was placed in the insulating cup and the cells rapidly frozen by immersing the apparatus in liquid nitrogen (-196 C). The cells were cleaved beneath the surface of the liquid nitrogen with a razor blade cooled to liquid nitrogen temperature. The lid was submerged in liquid nitrogen, placed over the cleaved

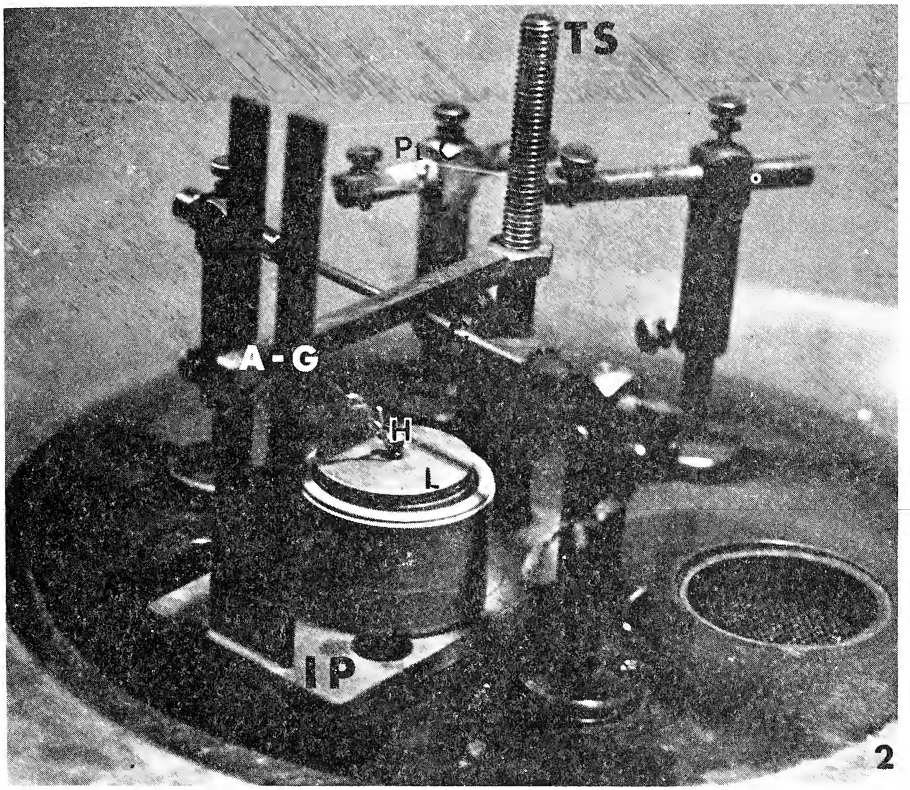


Figure 2. Photograph of the apparatus as it appears on the insulating platform (IP) of the vacuum evaporator. The lid (L) covering the cleaved cell surface is attached to the arm-guide (A-G) by a hook (H), and the lid is raised by rotation of the threaded shaft (TS). The Pt-C and C electrodes are also apparent.

cell surface, and the entire apparatus quickly transferred to the insulating platform inside a Mikros VE 10 vacuum evaporator (Mikros, Inc., Portland, OR). During evacuation, frost forms on the top of the lid and on the sides of the insulating cup, but not on the surface of the cells in the holding block. The cleaved surface was etched by withdrawing a thin block of insulating material, separating the insulating platform and insulating cup, with a length of fine cord, and then adjusting the lid to a position 0.1-0.2 cm above the cleaved cell surface. In this way the temperature of the block is increased gradually, and the cleaved cells surface etched by the slightly raised, and colder, lid. The method is unsophisticated, the temperature of the block and lid are not monitored, and etching times must be empirically determined, with periods between 3-12 min routinely employed.

After the cells were etched, they were shadowed with Pt-C (open arrows, Figure 3) at an angle of approximately 45°, and backed at 90° with C (solid arrow, Figure 3) for support. The chamber was pressurized and the holding block allowed the

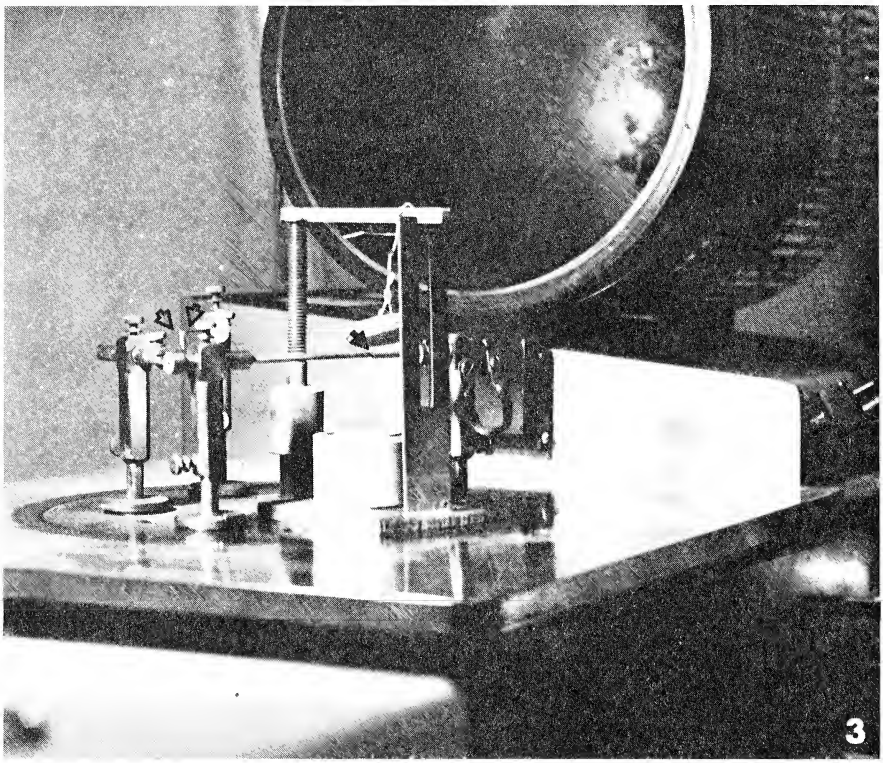


Figure 3. Photograph of the apparatus as it appears during etching and replication, bell jar removed. After a vacuum of $10^{-5}$ Torr is achieved, the lid is raised 0.1-0.2 cm, the cell surface etched, and the lid raised approximately 8.5 cm to permit unobstructed deposition of Pt-C (open arrow) and the C (solid arrow) by evaporation.

equilibrate to ambient temperature. Upon immersion in distilled water, the replica invariably broke apart. Replicas could be collected with less contamination by placing a thin plastic mask over the holding block, exposing only the cells which were frozen cleaved, and etched. The replica was treated for 1 hr in $H_2SO_4$, rinsed several times in distilled $H_20$, and treated for another hour in approximately 5% sodium hypochlorite (commercial Chlorox) to remove adhering cellular material. The replica was rinsed again in distilled $H_20$ and collected on a collodion-coated 300-mesh copper grid. All replicas were examined with an RCA-EMU-3G electron microscope at 50 kV. Photographic negatives were taken at initial magnifications of 7,700-11,500.

An aquatic *Bacillus subtilis* and a *Pseudomonas sp.* were cultured on trypticase soy agar for 18-24 hr at 30 C prior to freeze-etching.

## RESULTS AND DISCUSSION

Replicas of bacterial cells obtained with this technique (Figures 4 and 5) are similar to those obtained with other freeze-etch procedures (Nanninga, 1969; Cagle, *et al,* 1972). Figure 4 is a replica of *B. subtilis* cleaved obliquely along the cell wall (CW) and plasma membrane (PM), and through the cytoplasm (Cy).

Figure 4. Frozen-etched preparation of *B. subtilis.* A small portion of the ordered arrangement of the cell wall (CW) subunits is apparent (solid arrows), with areas of plasma membrane (PM) and cytoplasm (Cy) exposed. Strand-like extracellular material (EM) which may be polymer or artifact is observed. In some cases, deep etching also exposes a second layer of cells (open arrows). Marker = 0.5 $\mu$m (x81,000).

Several flagella (F) adhere to the cell wall of the organism and numerous strands of extracellular material (EM) are also apparent. A small portion of the ordered structure which comprises the cell wall is evident on the exterior of one cell (solid arrows), which has been previously reported as a surface component of numerous *Bacillus* species (Holt and Leadbetter, 1969). The plasma membrane (PM) contains particles, characteristic of this layer in frozen-etched preparations. The absence of temperature control results in deep etching, and some replicas reveal a second layer of cells (open arrows) (Figure 4).

A similar cleavage pattern is also observed with the aquatic pseudomonad, fractured to reveal a large portion of the cell wall (CW), and a small portion of the granular plasma membrane (PM) and cytoplasm (Cy) (Figure 5). The structure of the bacterial cell wall is partially observed (open arrows), although much of the wall is masked by eutectic material (solid arrows). The presence of this material indicates that the temperature of the holding block increased during etching, with melting of some low melting-point, salt-containing matrices.

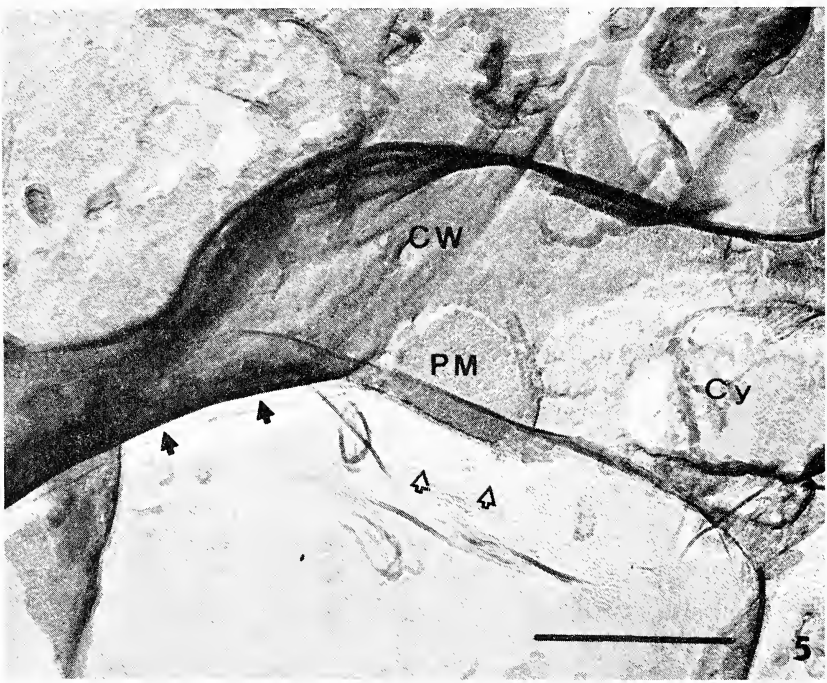


Figure 5. Freeze-etching of *Pseudomonas sp.* Eutectic material (solid arrows) masks most of the cell wall (CW) substructure (open arrows). Portions of the cell wall have been removed exposing the granular plasma membrane (PM) and the deeply etched cytoplasm (Cy). Marker = 0.5 $\mu$m (x53,000).

The freeze-etch device and procedures employed (Figures 1-3) combine the etching method introduced by Hall (1950) and Steere (1957) with the simple freeze-cleave apparatus of Bullivant and Ames (1966). The replicas in Figures 4

and 5 are representative of those which may be routinely obtained, although better results may be attained with practice. Cells cleaved through the cytoplasm appear to be adversely affected by the relatively long etching times, and the best replicas obtained were those cleaved along the plasma membrane, or tangent to the cells which are subsequently exposed by etching. Although replicas produced with this device do not always possess the fine structure which may be achieved with sophisticated freeze-etch devices, as the Balzers device (nor is it designed to replace the Moor device as a research tool), it does provide a means for introducing the students to the methodology of freeze-etching, as well as to some techniques of cryo-biology. The advantages of this freeze-etch device are its simplicity and low cost (less than $45.00); however, experience with the apparatus has shown that this method is highly empirical, and that successful replicas are not always initially obtained.

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# THE FLOWER GARDENS CORAL REEFS

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ABSTRACT

The Flower Gardens Coral Reefs, located 110 nautical miles SSE of Galveston Island, Texas are the northernmost living coral reefs in the Gulf of Mexico. Today, these reefs are an area of intense exploration, partly because of their ecological isolation and preservation of plant and animal life. Approximately 360 marine species, many previously unreported in the Gulf, have been identified within them. Also, marine invertebrates available at the reefs are animals ideally suited for comparative neurobiological studies. In addition, the West Flower Garden reef possess the most fully-developed reefal biotic communities along the Texas-Louisiana coast.

## INTRODUCTION

The Flower Gardens Coral Reefs (East and West), located approximately 110 nautical miles SSE of Galveston, Texas on the outer edge of the continental shelf were virtually unexplored, until recently. Today, partly because of their ecological isolation and preservation of marine plant and animal life, the reefs are the focus of intense exploration, much of it by scientists from the Marine Biomedical Institute (MBI) of the University of Texas Medical Branch at Galveston, Texas A&M University (TAMU), the Universities of Houston and Southwestern Louisiana and the National Museum of Natural History.

## WORK UNDERTAKEN

The "Miss Freeport" is a 135-foot research vessel (under operational control of MBI) which can support up to 21 diver-scientists, who usually work in depths from 70 to 85 ft. Occasionally they dived to 190 ft along the edge of the reef. Near the Great Escarpment, the 2-man submersible, "Nekton Gamma," (General Oceanographics, Inc., Newport Beach, California) carried scientists to depths exceeding 450 ft. The excursion defined the reefs diverse biological and geo-

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logical forms, and provided evidence refuting the earlier conclusion that the Flower Gardens coral reef was dead. It's alive, thriving and much deeper (more than 450 ft) and older than imagined. Moreover, it is the northernmost living coral reef in the Gulf of Mexico and to date some 360 marine species, many previously unreported in the Gulf, have been identified within it. Under the sponsorship of MBI, Drs. Thomas Bright and Linda Peguegnat of TAMU and their collaborators published a comprehensive survey of living forms on the reef; the book is entitled, "Biota of the West Flower Garden Bank" (Gulf Publ. Co., Houston, Texas). In addition, geologists and engineers edited a detailed bottom map. Scaled at one in/100 ft, the map's resolution exceeds those of the lunar surface compiled by NASA prior to the Apollo Missions. To quote Robert Alderdice, operation's officer in charge of the Flower Gardens Cruises, "Incredibly, man has more detailed information of another planetary body than its own coastal waters, which have been navigated for centuries."

The continental shelves of the United States possess a tremendous potential as new geographic support areas near the coastal zone and an ultimate goal is to make the shelves inhabitable. But, how to successfully integrate them into the nation's economic geography is still another problem. The solution requires, among other things, detailed maps and complete geological knowledge of the shelves. The Flower Gardens reefs, on the edge of the Texas Continental shelf probably formed originally atop sedimentary rock forced upward by a salt dome. Whether the coral is solid and continuous or only a cap over a substrate of rock is unknown, but core sampling may provide the answer.

Moreover, the Flower Gardens are a unique biological and chemical community, and in many respects it is an ecologically closed environment. The water is remarkably clear (to around 200 ft), always between 21 C to 22 C (70 F to 72 F) and much of the indigenous flora and fauna are found nowhere else in the Gulf. Furthermore, the West Flower Garden coral reef has the most fully-developed series of reefal biotic communities along the Texas-Louisiana coast and studies here may furnish answers applicable to other biotic structured communities. The crest of the reef itself is approximately 75 ft beneath the water's surface. The depth across most of the reef is 70 to 85 ft, while surrounding waters are 350 to 460 ft deep. Approximately 40% of the lower 80 acres of reef atop the West Flower Garden is covered by viable building corals, primarily species of *Diploria, Montastrea* and *Porites*; the massive coral heads approach 10 ft in diameter. These spectacular coral growths, plus communities of sessile and mobile organisms attract divers and undersea photographers during spring and summer. Myriad species of fish and hundreds of invertebrate varieties are encountered. Commercial snappers (*Lutjanus sp.*) and groupers (Family Serranidae) occupy the deeper flanks of the Flower Gardens reefs which are frequented by fishing boats. Utilizing submersibles, underwater television and other appropriate techniques, investigators hope to observe migration, schooling reproductive behavior, habitat preference and feeding characteristics of the snappers and other commercial and sport fishes.

Other organisms found at the Flower Gardens include foraminifers, sponges (Porifera), marine worms (Polychaeta), brachipods, numerous varieties of gastropods, mollusks, crustaceans and echinoderms. There are over 100 species of tropical fish. Particularly fascinating on the living reef above 150 ft are the diverse polychaetous worms. Some worms (*Eunice sp.*) are exceptionally large with one specimen of *E. aphroditois* nearly 2 ft long. Echinoderms occupy all depths of the West Flower Garden bank, while a sizeable crustacean population occurs between 150 and 280 ft. Among the bottom fish, *Chromis enchrysurus* is most numerous. The grouping of exclusively deep reef fishes is intriguing and contains the most important commercial food fish in the Gulf of Mexico. The Red snapper (*Lutjanus campechanus*) and several relatives, including the Vermillion snapper (*Rhomboplites aurorubens*). Catches of Red snapper are better during fall and winter, but in June, 1972, considerably more Red than Vermillion snapper were observed over the drowned reef and additional investigation of the feeding behavior of these 2 species appears warranted. Also significant were the occasional sightings of the rather rare serranid, the Spanish flagfish, (*Gonioplectrus hispanus*) and the butterfly fish, (*Chaetodon aya*), the latter previously reported absent. Further descriptive and quantitative data regarding the biota of deeper portions of the West Flower Garden bank and other reefs should clarify the complex inter and intracommunity relationships.

Virtually all exploration of the Flower Gardens reefs involved putting marine scientists into the sea, they "went after" their subjects of study. This is quite remarkable as "traditional oceanography" has been a science of remote control using dredges, pumps and elaborate instrumentation usually manipulated from the deck of a ship. The direct approach of "diver oceanography" is more dependable; it produces extremely accurate scientific data and is less harsh to the ecosystem.

Environmental studies acquire high-priority from marine scientists. The Gulf of Mexico receives nearly 2/3 of the sediment and industrial waste from the continental United States and over 3,000 offshore oil wells and miles of subsea pipelines dot the coastal waters of Texas, Louisiana and Mexico. The concentration of chlorinated hydrocarbons and heavy metals in organisms from the Flower Gardens is somewhat higher than from other Gulf areas. By performing environmental quality baseline studies, scientists hope to create a continuous program of biological sampling and monitoring.

In addition, medicine can learn much from the sea. And, comparative neurobiology offers infinite possibilities for medicine. The elaborate neuronal organ system of mammals, including man, is fashioned upon structures and principles evolved over millions of years, primarily in the sea. The chemical transmitters, hormones and enzymes in man also occur in ancient, less complicated marine invertebrates. These chemical substances vary between species, but their fundamental molecular structure is identical in almost every respect. Similarly, specialized tissues such as neurons are nearly identical in marine organisms and man. Medicine demands an understanding of how human beings adapt to their environment, and the brain, with its neurons and trillions of circuits is the chief

regulator of adaptability. Study of simpler nerve networks and untangling the complexities of neural integration and transmission in organisms such as the marine mollusk, *Aplysia brasiliana*, a shell-less sea snail whose brain is conveniently located in its belly and contains approximately 1,500 neurons, is a first step in penetrating the circuitry of man's brain. Thus, tapping the sea's resources will harvest new knowledge prerequisite to solving many of today's environmental and medical problems. Indeed, the sea is man's final frontier on earth.

# A REEVALUATION OF TWO NEW SPECIES OF FOSSIL BATS FROM INNER SPACE CAVERNS

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ABSTRACT

Choate and Hall (1967) proposed 2 new species of *Myotis* on the basis of a small collection of Pleistocene fossils recovered from Inner Space Caverns, Georgetown, Texas. Since that time a large number of topotypes have been recovered and as a result a reevaluation of the 2 extinct species was undertaken. One, *M. magnamolaris* is considered conspecific with the living *M. velifer* and the other, *M. rectidentis* is considered valid.

## INTRODUCTION

Pleistocene bat remains from Inner Space Caverns (formerly Laubach Cave) in Georgetown, Texas, were first reported by Slaughter (1966) and referred to as "*Myotis* sp." This small collection was later studied and reported on by Choate and Hall (1967) and 2 new species were proposed, *Myotis magnamolaris* and *Myotis rectidentis.*

The cave assemblage, is believed to have accumulated during the last major interstadial, probably between 25,000 and 40,000 years before the present. A radiocarbon date on bones of the extinct peccarry, *Platygonus,* produced an age of 13,900 ± 400. Considering the stage of bone dating at that time, this is considered to be a minimum date and Slaughter believes the bones to be in excess of 20,000 B.P.

Choate and Hall's study (1967) was based on 23 partial and complete mandibles and 2 left maxilli. Since that time the cavern has been revisited and an additional 79 mandibles and 6 maxilli were recovered. These 2 collections are combined for a reevaluation of the 2 species. Forty-seven specimens of modern *Myotis velifer incautus* were also included in the study for comparative purposes. Measurements included in the table and discussions are in mm and were taken by means of an ocular micrometer in a binocular microscope.

### *Myotis rectidentis* Choate and Hall (1967)

*Referred Specimens*

Holotype (SMU-SMP 61780); 10 mandibles including the paratype and new topotypes. All specimens are from Inner Space Caverns, Georgetown, Travis County, Texas.

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*Comparison With Other Species*

Considerations for comparative species were based on the modern geographic ranges of the various species.

*M. rectidentis* can be distinguished from *M. yumanensis* by the smaller size of the latter.

*M. lucifugus occultus* is about the size of *M. rectidentis*, but the premolars are more crowded.

Finally, *M. keenii apache* has a thinner mandible with a straight shape in contrast to the curving shape of the mandible of *M. rectidentis.*

*Discussion*

Morphologically, *M. rectidentis* seems most like modern *M. velifer* but scatter diagrams showing the length-width ratios of m1, m2, and m3 place the holotype of *M. rectidentis* and 9 other specimens in a distinct cloud from both *M. magnamolaris* and modern *M. velifer.* As the results of the molar diagrams are identical, only the one for m2 is presented (Figure 1). Only 2 lower canines are present in the collection so they were not plotted. With canine lengths of 1.25 and 1.5, they were smaller than those of *M. velifer.* No significant differences other than size were found between *M. rectidentis* and *M. velifer.* Nevertheless, the size is so

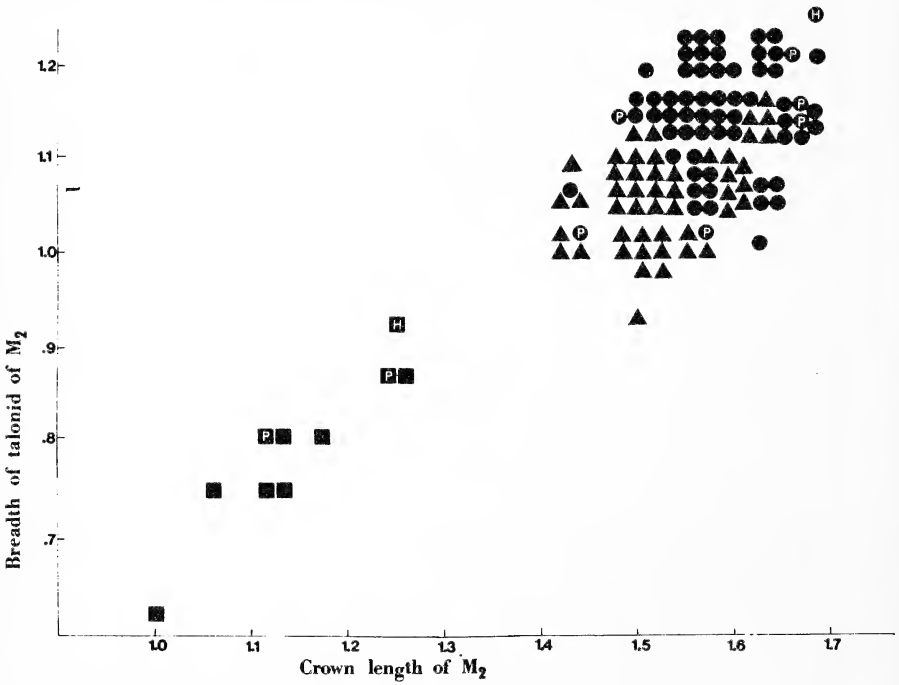


Figure 1. Scatter diagram of the length-width ratio of m2. Triangles represent modern *M. velifer*, squares represent fossils of *M. rectidentis*, and circles represent fossils of *M. velifer* from Inner Space Caverns. the holotypes of *M. rectidentis* and *M. magnamolaris* are designated H, paratypes P, and topotypes are the unlettered symbols.

consistent, it is felt that the species is valid, and it is agreed that *M. rectidentis* is a bat "similar to *M. velifer* but smaller." The *M. velifer* sample included almost an equal number of males and females, eliminating the possibility that the 2 clouds on the scatter diagram represent sexual dimorphism.

*Measurements*

For means and extremes of lower teeth see Table 1. There are 4 specimens containing upper molars that fit in with the smaller *M. rectidentis* species. One such specimen was described by Choate and Hall (1967) while the other 3 were more recent finds at the cave. The new maxilli have crown lengths of M1 ranging from 1.37 - 1.62, of M2 ranging from 1.37-1.56, and of M3 ranging from 0.75-0.81. The greatest width of the molars varies from 1.31 - 1.93 in M1, from 1.37 - 1.56 in M2, and the lone measurement of M3 is 1.37. These measurements are smaller than similar measurements of *M. velifer.*

TABLE 1

| | Lower molar tooth row | m1 | | m2 | | m3 | |
|---|---|---|---|---|---|---|---|
| | | Length | Width | Length | Width | Length | Width |
| Modern velifer | 4.31-4.68 | 1.43-1.62 | 1.00-1.18 | 1.43-1.56 | 0.93-1.18 | 1.31-1.56 | 0.87-0.93 |
| Means | 4.47 | 1.53 | 1.08 | 1.51 | 1.05 | 1.41 | 0.90 |
| SMU Fossils | 4.25-5.06 | 1.50-1.75 | 1.06-1.37 | 1.43-1.68 | 1.00-1.25 | 1.06-1.62 | 0.68-1.06 |
| Means | 4.64 | 1.60 | 1.16 | 1.57 | 1.07 | 1.46 | 0.95 |
| *M. rectidentis* | 3.12-3.68 | 1.12-1.31 | 0.75-0.87 | 1.00-1.25 | 0.62-0.93 | 0.87-1.12 | 0.50-0.75 |
| Means | 3.42 | 1.20 | 0.83 | 1.15 | 0.80 | 1.04 | 0.65 |

*Myotis velifer* (J. A. Allen)

*Myotis magnamolaris* Choate and Hall (1967)

*Referred Material*

Sixty-eight mandibles, 4 maxilli, and 1 complete palate from Inner Space Caverns, Georgetown, Travis County, Texas. This includes the holotype (SMU-SMP 61772) and paratypes of *M. magnamolaris.*

*Discussion*

Choate and Hall's (1967) diagnosis of *M. magnamolaris* placed greatest significance on the large size of the fossil form. Length-width ratios were plotted on scatter diagrams for m1, m2, and m3, for the holotype, paratypes, for the greatly expanded collection of topotypes, and finally for the specimens of modern *M. velifer* (Figure 1). As the results were exactly the same for each molar, only the diagram of m2 is presented. The fossils from Inner Space Caverns and the modern sample form a continuous cline, although the holotype of *M. magnamolaris* is the largest of the collection.

Choate and Hall's (1967) diagnosis also characterizes *M. magnamolaris* as having a lower canine that is massive and long. Only one canine (the holotype's) however, was preserved in the original collection (see Figure 2). We now have several others and the size of the canine seems to be a matter of allometric growth, considering the greater size of the holotype. No other morphological differences were found in the fossils that cannot be duplicated in modern *M. velifer.*

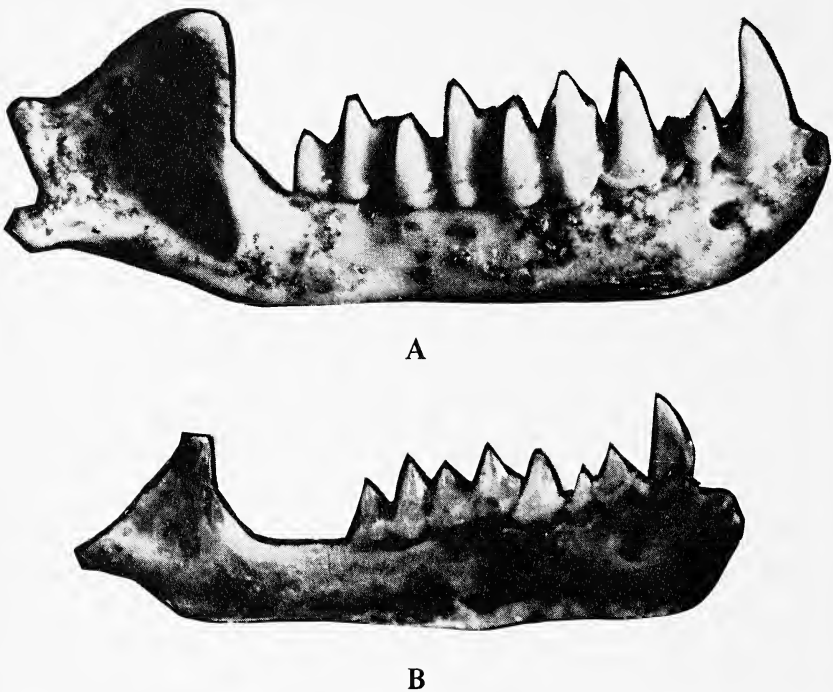


Figure 2. View of the tooth row of the holotypes of (A) *M. magnamolaris* and (B) *M. rectidentis.*

It seems possible that the Inner Space bats represent a rather basic group for the species and may actually be ancestral to one or more modern subspecies. Choate and Hall (1967) suggested that the fossils are "possibly the direct ancestor of *M. velifer.*" It seems equally possible, however, that the fossils represent an extinct geographic subspecies that on an average was a bit larger than the modern myotids. It is known that many extant species were larger in size during the late Pleistocene. In any case, only maximum sized specimens of *M. magnamolaris* could be distinguished from *M. velifer.*

If, of course, additional material (i.e., skulls) should provide viable characters allowing separation of the form from the modern form, it would be of no more than subspecific rank. Whether it proved to be an extinct geographic race, a dead-end temporal race, or a race ancestral to one or more modern subspecies, it would be proper to be designated as *M. velifer magnamolaris* Choate and Hall (1967).

*Comparison With Other Species*

*M. volans* has anterior premolars that are crowded out of the anterior-posterior tooth row as in the fossil *M. velifer,* but the protoconid basin of P4 is constricted both anterior and posterior to form a barbell-shaped basin. Also the hypocone is not as prominent a cusp as in the fossil forms.

*M. thysanodes* differs from the fossil *M. velifer* in several ways. First, there is no crowding of the anterior premolars, also there is a weaker development of the crest that extends from the protocone-hypocone to the lingual face of the metacone. Finally, the protocone extends farther lingually than the hypocone, while in the fossil forms the protocone and hypocone are subequal, giving it a more quadrangular appearance.

*Measurements*

For means and extremes of lower teeth, see Table 1. In the way of maxillary measurements there are 4 specimens including a complete palate in addition to the previously described maxillary from 1967. The crown-length range of M1 is from 1.88-1.75, in M2 from 1.62-1.68, and in M3 from 0.93-1.12. The greatest breadth of M1 is from 1.62-1.8, of M2 from 1.75-1.93, and of M3 from 1.37-1.81. These fall well within the range of modern velifer.

## SUMMARY

The collection of bat fossils from Inner Space Caverns (formerly Laubach Cave) has been greatly expanded. A reevaluation of the 2 extinct species proposed by Choate and Hall (1967) was undertaken as a result. The following conclusions have been reached.

*M. rectidentis* Choate and Hall (1967) cannot be distinguished from *M. velifer* on any morphological basis other than by its smaller size. Scatter diagrams of the length-width ratios of the molars demonstrate that the size range does not overlap with either modern Texas myotids or those from Inner Space previously described as *M. magnamolaris.* I therefore feel that this species is valid.

The holotype and paratypes of *M. magnamolaris* form a continuous cline, with respect to size ratios of the teeth with the modern species of *M. velifer* and the newly collected topotypes from Inner Space Caverns. *Myotis magnamolaris* is therefore considered conspecific with *M. velifer.* The fossil population may average slightly larger than the modern population, but 95% of the specimens could not be distinguished from the modern form. The fossils could represent an extinct geographic race, or a temporal race ancestral to one or more modern subspecies.

## ACKNOWLEDGEMENTS

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the recent *Myotis velifer* collection and Dr. Henry Setzer, U.S. Natural History Museum, for allowing my study of the collections under his care.

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# SEASONAL DISTRIBUTION OF MITES ASSOCIATED WITH *POPILIUS DISJUNCTUS* (ILLIGER) (COLEOPTERA; PASSALIDAE) IN HARDIN COUNTY, TEXAS

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ABSTRACT

Seasonal distribution of mites associated with *Popilius disjunctus* was studied by collecting 2 sets of beetles, 1 in the winter and 1 in the summer of 1974, from a beech–magnolia forest in Hardin County, Texas. Some correlation between climatic conditions and mite population/beetle was noted. The region being temperate–subtropic, the seasonal variation in temperature was minimal and thus had an effect on mite population density and kinds present.

## INTRODUCTION

*Popilius disjunctus* (Illiger), as described in many works, is a semisocial beetle found in rotting oak, hickory, gum, beech, and magnolia logs. It has been utilized by several researchers for both laboratory and field experiments and observations (Borror and DeLong, 1954; Pearse, *et al.*, 1936; Gray, 1946; Hunter and Mollin, 1964; Harrel, 1967). Work on the seasonal distribution of the commensal and parasitic mites associated with *P. disjunctus* has been limited to relatively few researchers. Hunter and Mollin (1964) and Harrel and Mathis (1965) have dealt specifically with seasonal influences on mite populations on *P. disjunctus* in North Carolina and Oklahoma. No work is known for the southeastern portion of Texas.

To further investigate this symbiotic relationship under seasonal influence and originate a study in southeast Texas, 2 collections of beetles were made in a post climax beech-magnolia forest in Hardin County, Texas, during the months of August and December, 1974.

## METHODS

On August 30, 1974, and December 22, 1974, 25 beetles were collected in a beech-magnolia forest in Hardin County, Texas, just off Highway 96. The mites were removed from 10 randomly chosen beetles and placed in 80% isopropyl alcohol.

When all mites on the exterior had been found and identified, the elytra and hind wings were removed to search for *Zercon passalorum* and *Heterochelytus*

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*fusiformis.* These 2 species of mites are believed to be the only 2 parasitic species found on the beetle (Pearse, *et al.,* 1936). In addition, numerous Hypopi (nymphs) (Family: Tyroglyphidae) were encountered in clusters under the elytra.

Identification was made on all mites, except *Cosmolaelaps passali* and *Oppelia* sp., by comparison with specimens identified by Dr. Preston Hunter. *C. passali* was identified utilizing a work by Hunter and Mollin (1964). *Oppelia* sp. was identified through personal communication with Dr. G.W. Wharton at Ohio State University in March, 1975.

When necessary, slides were made of each of the species found, particularly of the 3 species of the genus *Uroobovella.* The *Uroobovella* sp. were not separately identified for correlative purposes by either Pearse, *et al.* (1936), or Harrel and Mathis (1965).

## RESULTS

Results are presented in Table 1. The average number of each species, where applicable, is compared to corresponding month averages obtained by Pearse, *et al.* (1936), indicated by an asterisk (*), and to the March averages of Harrel and Mathis (1965), identified by a double asterisk (**).

A total of 20 beetles were examined. The total number of mites found in August, 1974, was 1858. In December, 1974, 3173 mites were found. Average mites/beetle was 185.8 and 317.3 respectively. A total of 14 mite genera were found, although *Oppelia* sp., *C. passali,* and *Caelonopsis latus* were found only in the August collection.

## DISCUSSION AND CONCLUSIONS

As indicated by Pearse, *et al.* (1936), Hunter and Mollin (1964), and Harrel and Mathis (1965), the species averages are highly variable. However, winter averages are somewhat higher than summer averages. This is especially true for *Zercon passalorum* and *Heterochelytus fusiformis.* Variations in results when compared to previous individual patent leather beetle works may be explained by many factors. Environmental conditions of Southeast Texas are unique. The 2 other studies used here for comparative purposes were done in North Carolina and Oklahoma. Those areas, while considered temperate in climatic conditions, are subjected to much more severe winters than the temperate-subtropic climatic conditions of Southeast Texas.

During the study the difference in the August and December temperature was not extreme. This held true of the whole winter, but overall climatic conditions for the area were not atypical to normal seasonal change. It is concluded that the increase in the number of mites found in the second collection (December) was due to the somewhat cooler climatic conditions and availability of habitat provided to mites when the beetles become more colonial during the winter months.

TABLE 1

Number of mites on *Popilius disjunctus*.
Top number is August, 1974, figure; bottom number is figure for December, 1974.

| Species | Beetle Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Avg. | * | ** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Heterochelytus* | 2 | 2 | 6 | 6 | 8 | 13 | 4 | 4 | 1 | 8 | 5.4 | 2.2 | 2.0 |
| *fusiformus* | 7 | 4 | 9 | 8 | 8 | 22 | 7 | 9 | 3 | 14 | 9.1 | 8.6 | --- |
| *Zercon* | 7 | 12 | 25 | 6 | 7 | 170 | 5 | 3 | 16 | 108 | 35.9 | 7.8 | 39.8 |
| *passalorum* | 14 | 15 | 161 | 130 | 19 | 132 | 130 | 88 | 22 | 147 | 85.8 | 81.4 | --- |
| *Macrocheles* | 4 | 7 | 32 | 5 | 2 | 1 | 13 | 6 | 26 | 15 | 11.1 | --- | --- |
| *tridentatus* | 4 | 5 | 20 | 43 | 7 | 13 | 12 | 37 | 19 | 21 | 18.1 | 0.9 | --- |
| *Urooborella* | 7 | 9 | 5 | 0 | 0 | 0 | 0 | 19 | 4 | 2 | 4.6 | --- | 71.6 |
| *spinosa* | 9 | 15 | 7 | 2 | 7 | 3 | 0 | 9 | 14 | 0 | 6.6 | 16.6+ | --- |
| Hypopi (nymph) | 48 | 36 | 295 | 40 | 23 | 552 | 38 | 19 | 44 | 89 | 118.4 | 2.4 | --- |
| (Tyroglyphidae) | 72 | 93 | 281 | 210 | 372 | 19 | 28 | 473 | 86 | 186 | 182.0 | 41.8 | --- |
| *Uroseius* | 4 | 0 | 5 | 0 | 2 | 0 | 4 | 5 | 10 | 3 | 3.3 | 9.9 | 2.7 |
| *quercus* | 2 | 3 | 0 | 7 | 2 | 10 | 1 | 0 | 5 | 6 | 3.6 | 6.5 | --- |
| *Cosmolaelaps* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | --- | 4.9 |
| *passali* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | --- | --- |
| *Urooborella* | 0 | 0 | 3 | 0 | 4 | 0 | 0 | 0 | 1 | 10 | 1.8 | --- | --- |
| *levis* | 0 | 7 | 4 | 0 | 3 | 2 | 0 | 0 | 3 | 0 | 1.9 | --- | --- |
| *Megathanus* | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 7 | 0 | 0 | 1.1 | --- | --- |
| *floridanus* | 2 | 0 | 6 | 0 | 5 | 0 | 0 | 3 | 2 | 0 | 1.8 | --- | --- |
| *Urooborella* | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 0.5 | --- | --- |
| *setosa* | 0 | 2 | 1 | 1 | 3 | 1 | 0 | 4 | 0 | 0 | 1.2 | --- | --- |
| *Caelonopsis* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.1 | --- | 3.0 |
| *latus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0.8 | --- |
| *Oppelia sp.* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | --- | --- |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | --- | --- |
| *Passalacarus* | 0 | 1 | 0 | 2 | 2 | 0 | 2 | 0 | 3 | 1 | 1.1 | --- | 5.8 |
| *sylvestris* | 4 | 3 | 2 | 0 | 1 | 7 | 6 | 0 | 8 | 1 | 3.1 | 1.5 | --- |
| *Seiodes* | 1 | 3 | 2 | 1 | 0 | 0 | 5 | 3 | 1 | 7 | 2.3 | --- | --- |
| *trifidus* | 5 | 7 | 2 | 0 | 3 | 7 | 6 | 8 | 1 | 1 | 4.0 | --- | --- |
| Totals | 73 | 70 | 374 | 61 | 53 | 736 | 74 | 66 | 106 | 245 | 185.8 | 32.0 | 129.8 |
| | 119 | 154 | 493 | 401 | 430 | 216 | 190 | 631 | 163 | 376 | 317.3 | 158.1 | --- |

* August average for Pearse, *et al.*, (1936) = Top number
December average for Pearse, *et al.*, (1936) = Bottom number
** March average for Harrel and Mathis (1964)
\+ The species of *Urooborella* not listed separately

The forest composition did not seem to have any correlative significance to the study, but further study is indicated to determine if tree specificity is relative to mite density and genera present.

Further research is also necessary to determine external body surface and subelytral subwing mite populations during a noticeable extreme of seasonal temperature in Southeast Texas.

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# CROWN POSITIONS WITHIN UNTHINNED LOBLOLLY PINE PLANTATION CANOPIES

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ABSTRACT

Crown class percentages are not affected by age or trees/acre. The percentage of dominant and suppressed trees is affected by land productivity.

## INTRODUCTION

Thinning practices employed to grow trees for selected products are usually defined by designating proportions of trees to be removed from each of the 4 main recognized crown classes. There is little available published information, however, regarding the distribution of tree crowns within the canopy prior to thinning.

Information collected from 219 unthinned old-field loblolly pine (*Pinus taeda* L.) plantations in the Interior West Gulf Coastal Plain during a mensurational study provided an opportunity to describe the effects of age, site index, and trees/acre on crown classes (Lenhart, 1972).

### *Plantation Measurements*

Plantations selected for sampling were unthinned, at least 9 yrs old, and undamaged by fire, insects, or disease. Within each plantation mensurational information, including age, site index (base age = 25), and trees/acre, were collected from a sample plot. In addition, the trees (10 to 16) on a sub-sample within the plot were each classified into one of 4 crown positions within the canopy—dominant, co-dominant, intermediate, or suppressed. These crown positions were converted to percentages.

### *Crown Class Percentages*

For all sample plots, the average % of trees in each crown class was as follows:

---

[1] Associate Professor and Assistant to the Dean.
[2] Former Graduate Research Assistants.
[3] Presently employed by Ministry of Communications in Taiwan.
[4] Presently employed by Drummond Coal Company.

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| Crown Class | Percentage |
|---|---|
| Dominant | 31 |
| Co-dominant | 28 |
| Intermediate | 31 |
| Suppressed | 10 |

The paucity of suppressed trees could suggest that many trees of this class have already died and disappeared. More probably, however, the spacing of these plantations—mostly nominally 6 X 8 feet—has been sufficient to support stands in which few trees have not yet been crowded into the suppressed condition.

*Crown Class Percentages by Age Classes*

Since the sampled plantations ranged in age from 9 to 30 yrs, we were able to describe crown positions in relation to 5-yr age classes (Figure 1).

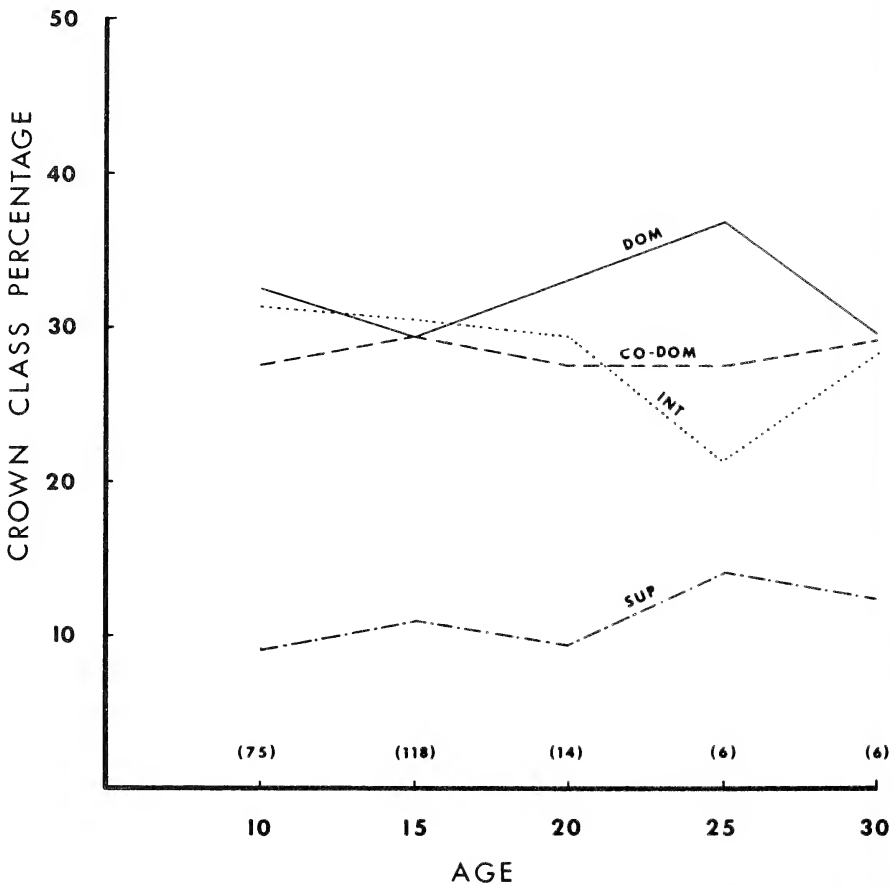


Figure 1. Percentage of trees in the 4 crown classes by age classes. Number of sample plots for each age class is shown in parentheses.

In age classes 10, 15, and 20, which included 95% of the sample plots, the % of crowns in each class is fairly constant. In these age classes, less than 6 %-points separate the upper 3 crown classes. Across all age classes about 10% of the trees had suppressed crowns. There was no indication of much shifting of trees between crown classes as they grow older.

*Crown Class Percentages by Trees-Per-Acre Classes*

The relationships between relative tree frequency in the 4 crown positions and trees/acre are shown in Figure 2. The sampled plantations ranged from 200 to 1700 trees/acre with 1 sample each in the 1400 and 1700 classes. About 92% of the sample plots were in the 400- to 1000-trees/acre classes.

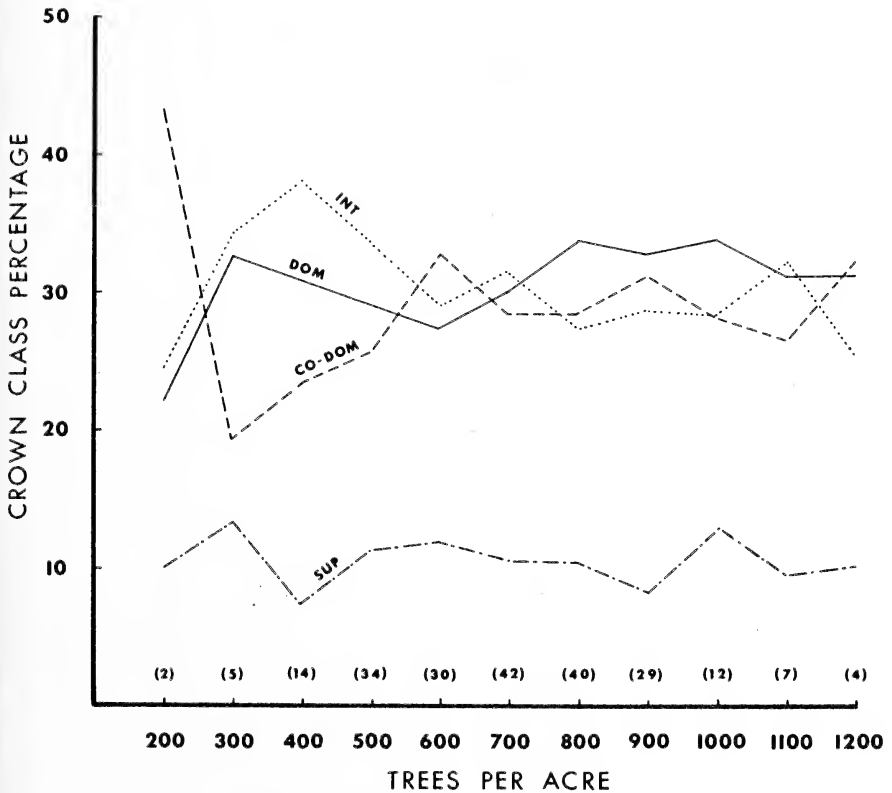


Figure 2. Percentage of trees in the 4 crown classes by trees/acre classes. Numbers in parentheses represent the sample size for each trees-per-acre class.

Trends in Figure 2 resemble those in Figure 1; each of the upper 3 crown classes includes about 30% of the trees, while the suppressed class represents about 10%. Between 600 and 1100 trees/acre about 4 to 7 %-points represents the spread between the dominant, co-dominant, and intermediate crown classes. No trends toward change in crown class with density are apparent.

*Crown Class Percentages by Site Index Classes*

Figure 3 depicts crown positions in relation to 5-ft site index classes. About 91% of the sampled plantations had site index values between 45 and 65 ft, inclusive.

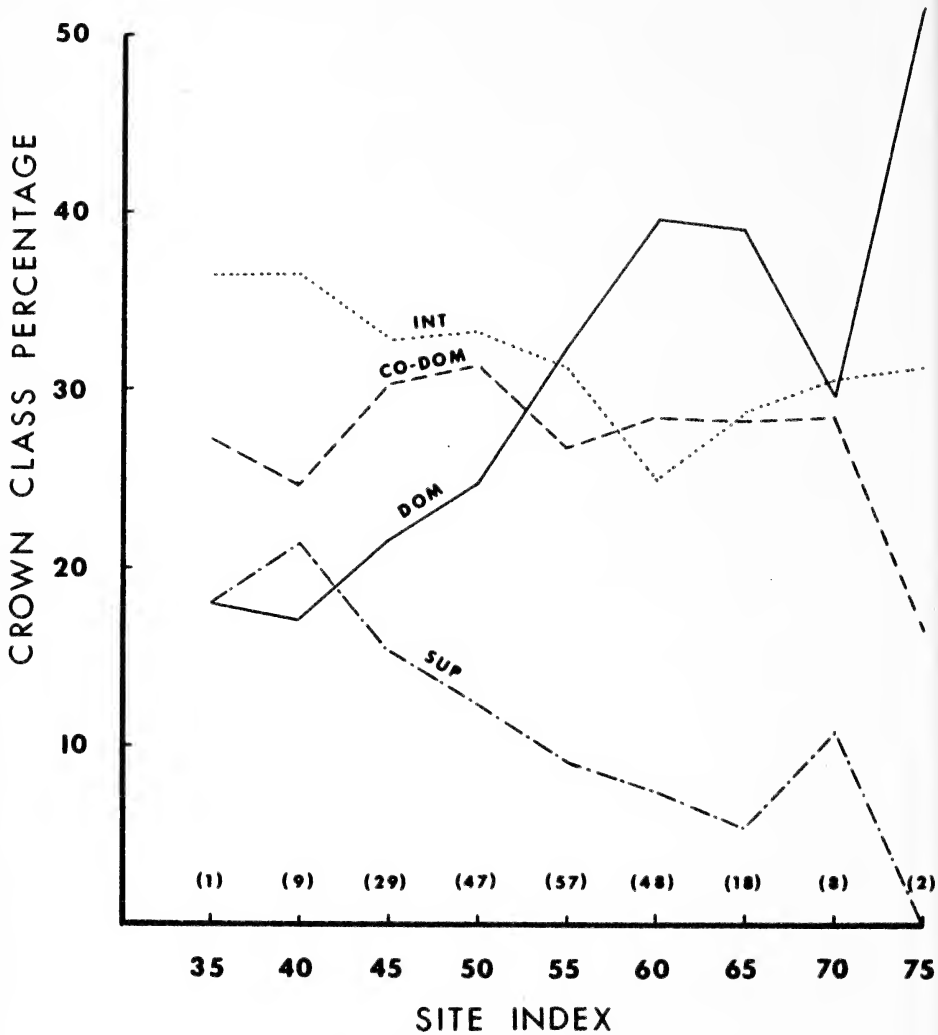


Figure 3. Percentage of trees in the 4 crown classes by site index classes. Numbers in parentheses represent the sample size for each site index class.

The proportions of trees in the top and bottom crown positions, definitely tend to vary with site index. As site index increases, the dominant % increases and the suppressed % decreases. Also, intermediate % appears to decrease slowly with increasing site index, while co-dominant % shows no definite trend.

On the poorer sites—35, 40, and 45 ft, about 40% of the trees are either dominant or suppressed. On the better sites—65, 70, and 75 ft, about 40% of the trees

are dominant, and 1 in 10 or less is suppressed. Across all site index classes, except 75 ft, the majority of the tree crowns are in either the co-dominant or intermediate classes.

The data suggest that in unthinned plantations within the ranges observed, the forester cannot expect any change in canopy composition as age or trees/acre increase or decrease. It may require some type of thinning regime to increase, for example, the % of dominant trees in a plantation. On the other hand, the % of dominant trees is larger and the % of suppressed trees is smaller on more productive than on less productive land. On the better sites about 40% of the trees are dominant.

## ACKNOWLEDGEMENTS

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This paper is based in part on a masters thesis by Mr. Ho.

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# PRODUCTION OF AMINO ACIDS BY SOIL MICROORGANISMS

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## INTRODUCTION

At the present time there is a revival in the use of microorganisms as agents for agricultural soil fertilization (Voznyakouskaya, 1963; Azcon, *et al.*, 1973). The basis for this practice resides in the knowledge that many soil microorganisms excrete amino acids, auxins, vitamins, etc., and that these substances enhance plant growth, either singly or in combinations showing synergistic effects (Vagnerova and Vancura, 1962; Brown, 1974). It is assumed that some microorganisms enhance plant growth better than others and that these can be identified even in the soil milieu.

This is a report on the production of specific amino acids by soil bacteria. The research objective was to determine the numbers of microorganisms in the soil which secrete amino acids and to assess the relative quantities produced. It is assumed that the data presented will serve to identify those organisms that can be used most effectively in commercial agricultural applications and perhaps even in the commercial production of amino acids.

## MATERIALS AND METHODS

### *Microorganisms*

The production of amino acids was studied in the laboratory using 171 different organisms isolated from soils from the provinces of Barcelona and Alicante in Spain. Assays of amino acids produced were performed using *Leuconostoc mesenteroides* ATCC 8042 auxotrophic for arginine, aspartic acid, glutamic acid, leucine and lysine while the production of alanine was monitored with *Pediococcus cerevisiae* ATCC 8081.

### *Media*

The soil bacteria studied were isolated and maintained on medium consisting of glucose, 20 g; $NH_4Cl$, 7 g; $MgSO_4 \cdot 7H_20$, 0.5 g; $KH_2PO_4$, 2 g; agar, 11 g; and

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enough water to make 1 l. the pH was adjusted to 7 before sterilization. Stock cultures of the auxotrophic organisms were maintained on Lactobacillus Broth AOAC (Difco) supplemented with the required amino acid. Production of amino acids was measured on specific amino acid assay media (Difco).

*Production of Amino Acids*

The assay for amino acids was based on the method used by Udaka (1960) for the isolation of glutamic acid-producing bacteria. This method was originally introduced by Steele, *et al.*, (1949) and more recently employed by Guinea (1970).

For this study, the auxotrophic organisms were spread on specific amino acid-deficient media and then soil isolates were streaked onto these seeded plates. Replicates were used to insure isolated and well-separated colonies. The plates were incubated at room temperature for 5 days and then evaluated as shown below.

Numerical value 0:
No growth of the auxotrophic organisms was observed macro- or microscopically.

Numerical value OM:
No growth of the auxotrophic organisms was observed macroscopically and only scant growth was seen microscopically. Growth was limited to the periphery of the colony of the amino acid-producing organism.

Numerical value 1:
Growth visible macroscopically; limited (1 to 2 mm) to the periphery of the colony of the amino acid-producing organism.

Numerical value 2:
A definite halo of growth surrounding the colonies of the amino acid-producing organisms. Growth extended some 3 to 4 mm from the colony and the auxotroph colonies were well developed. (Figures 1 and 2)

Numerical value 3:
Abundant, confluent growth surrounding the colonies and extending more than 4 mm from the colony.

While these criteria do not yield exact measurements, the growth patterns observed (Figures 1 and 2) are easy to assess for assignment of numerical values. Since several parameters are used, i.e., number of auxotroph colonies, density of growth, and extent of zone which supports growth, it is assumed that the numerical value is a measure superior to any single criterion.

*Evaluation of Amino Acid Production*

Each of the 171 soil isolates as assayed for production of alanine, arginine, aspartic acid, glutamic acid, leucine, and lysine.

The "index" of production of amino acids (P) by any given microorganisms can be determined by summing the numerical values; 0, 1, 2, 3 and dividing this sum by the number (n) of amino acids measured.

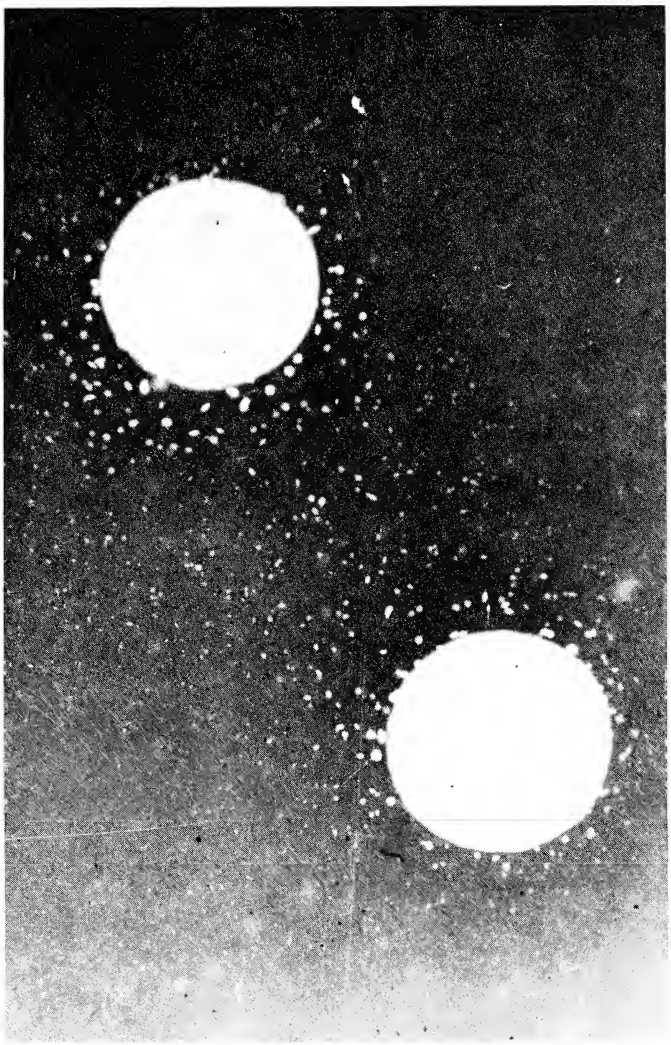

Figure 1. Growth of auxotrophic organism associated with amino acids excreted by soil bacteria.

In like manner, the "index" of production of amino acids by a group of microorganisms (e.g., Gram positive heterotrophs) can be obtained by dividing the sum of indices for each organism in the group by the number of microorganisms.

*Statistical Treatment*

In order to evaluate the significance of the differences (percentages) of organisms capable of producing a given amino acid, the following formula was employed.

$$k_1 = \frac{P_1}{n_1}; \quad k_2 = \frac{P_2}{n_2}$$

and:
$$k = \frac{P_1 + P_2 + \cdots P_x}{n_1 + n_2 + \cdots n_x}$$

then:
$$d = \frac{k_1 - k_2}{\sqrt{k(1-k)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where: $P_1$ = the index of production of amino acids by a given organism
$P_2$ = the index of production of amino acids by a second organism
$n$ = number of organisms tested

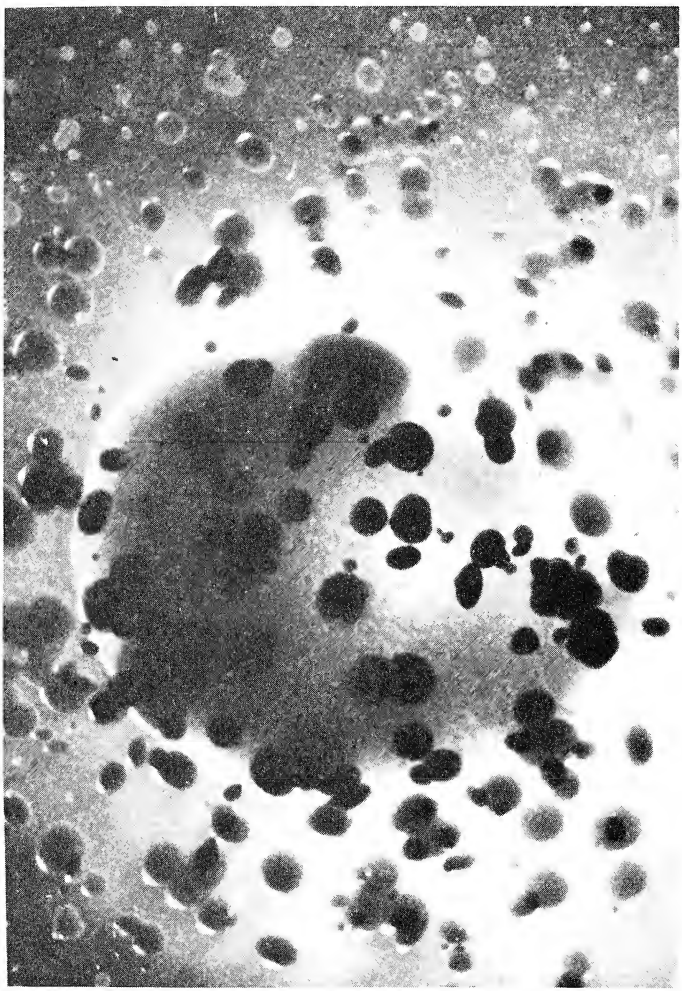

Figure 2. Microcolonies of auxotrophic organism surrounding colonies of amino acid-producing bacteria.

The calculated value of d was assumed to be derived from a population of values distributed normally about the mean and the value of 1.96 ($\alpha = 0.05$; 95% confidence level) was used as a measure of statistical significance. It was assumed that all values of d greater than 1.96 show statistically significant differences in the populations of soil bacteria capable of excreting specific amino acids.

In like manner, differences in indices of production of amino acids by these microorganisms or groups of microorganisms can be evaluated by the following equation:

$$Z = \frac{\bar{X} - \bar{Y}}{\sqrt{\frac{S_x^2}{n_x} + \frac{S_y^2}{n_y}}}$$

where: $\bar{X}, \bar{Y}$ = means of indices

$S_x^2, S_y^2$ = variance of the measured values

$n$ = number of values obtained

The value of $S^2$ was determined from the equation

$$S^2 = \frac{1}{n-1}\left[\Sigma(x^2) - \frac{1}{n}(\Sigma x)^2\right]$$

The number Z is also assumed to be derived from a normally distributed population; for $\alpha = 0.05$ the value 1.96 is used.

## RESULTS

The data shown in Table 1 indicate that numbers of organisms capable of excreting amino acids and the extent of excretion.

TABLE 1

Percentage of Bacteria Isolated from Soil
Which Secrete Amino Acids Into the Growth Medium

| Numerical value of production | Amino acids | | | | | |
|---|---|---|---|---|---|---|
| | Ala | Arg | Asp | Glu | Leu | Lys |
| OM, 1, 2, 3 | 91 | 87 | 88 | 79 | 94 | 69 |
| 1, 2, 3 | 83 | 47 | 76 | 42 | 74 | 43 |
| 2, 3 | 45 | 18 | 42 | 20 | 36 | 27 |
| 1 | 38 | 29 | 34 | 22 | 38 | 16 |
| 0 | 9 | 13 | 12 | 14 | 6 | 31 |

The data in Table 2 show the index of production of amino acids by 2 major groups of bacteria isolated from the soil. The data indicate different levels of production of each of the 6 amino acids studied.

TABLE 2

Index of Production of Amino Acids by Groups of Soil Microorganisms.

| Group of microorganisms | Index | | | | | |
|---|---|---|---|---|---|---|
| | Amino acids | | | | | |
| | Ala | Arg | Asp | Glu | Leu | Lys |
| Gram positive | 1.63 | 0.79 | 1.50 | 0.71 | 1.33 | 0.78 |
| Gram negative | 1.24 | 0.78 | 1.15 | 0.77 | 1.35 | 0.95 |

## DISCUSSION

*Analyses of Amino Acid Production*

The quantities of amino acids secreted by bacteria isolated from different soils vary over a wide range. For example, 94% of the organisms tested (161/171) showed some production of leucine. Seventy-four % showed production at levels 1, 2, and 3; 36% at levels 2, 3; and 18% at level 3 (Table 1). We infer from these data that although the majority of bacteria isolated from the soil secrete leucine, only a small number (31,171; 18%) secrete sufficient quantities to support extensive growth of other microorganisms. Analysis of the differences observed indicated that these differences are statistically significant at the 95% level of confidence (Table 3).

The data in Table 2 also show that alanine, aspartic acid, and leucine are produced in larger quantities than arginine, glutamic acid, and lysine. The difference between these 2 groups is statistically significant but the differences seen within each group (i.e., alanine-aspartic acid, alanine-leucine, aspartic acid-leucine, etc.) are not. The values for Z shown in Table 4 confirm this observation at the 95% confidence level.

When the values obtained were analyzed with respect to Gram reaction of the organisms studied, it was seen (Table 2) that both groups, Gram positive and Gram negative, secreted greater amounts of alanine, aspartic acid, and leucine than of arginine, glutamic acid, and lysine. This difference was statistically significant but the differences observed within each group were not. From this we infer that the type and quantity of amino acids secreted by Gram positive bacteria is not different from that secreted by Gram negative bacteria and that certain amino acids are secreted in relatively large quantities by both groups.

TABLE 3

Values of d for Comparison of Percentages of Bacteria Capable of Excreting Amino Acids

| Amino acid | d for difference* | | |
|---|---|---|---|
| | 0, 1, 2, 3 vs. 1, 2, 3 | 1, 2 vs. 2, 3 | 2, 3 vs. 3 |
| Alanine | 1.949 | 7.372 | 5.242 |
| Arginine | 8.105 | 2.698 | 0.901 |
| Aspartic Acid | 2.740 | 6.379 | 6.241 |
| Glutamic Acid | 6.302 | 4.315 | 3.176 |
| Leucine | 4.662 | 6.864 | 3.336 |
| Lysine | 2.732 | 2.709 | 2.915 |

*Values of d greater than 1.96 represent statistically significant differences at the 95% confidence level.

TABLE 4

Values for Z for Index of Amino Acid Production by all the Organisms Tested*

| | Ala | Arg | Asp | Glu | Leu | Lys |
|---|---|---|---|---|---|---|
| Ala | – | 6.548 | 1.088 | 6.651 | 1.220 | 4.869 |
| Arg | 6.548 | – | 4.802 | 0.400 | 4.513 | 0.707 |
| Asp | 1.088 | 4.802 | – | 5.226 | 0.161 | 3.672 |
| Glu | 6.651 | 0.400 | 5.226 | – | 4.882 | 1.065 |
| Leu | 1.220 | 4.513 | 1.161 | 4.882 | – | 3.435 |
| Lys | 4.869 | 0.707 | 3.672 | 1.065 | 3.435 | – |

*Values of Z greater than 1.96 represent statistically significant differences at the 95% confidence level.

## SUMMARY

A simple technique was used to study the secretion of alanine, arginine, aspartic acid, glutamic acid, leucine, and lysine by 171 different bacteria isolated from soils in the provinces of Barcelona and Alicante in Spain. Statistical analyses of the data obtained showed that a large portion of soil isolates excrete amino acids into the growth media but that only a much lower percentage secrete sufficient quantities to support abundant growth of auxotrophic bacteria.

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# THE BATS OF EAST TEXAS

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ABSTRACT

Information concerning the distribution of bats in East Texas is scanty at best. Bailey (1905), in his *Biological Survey of Texas,* recorded only 4 bats from east Texas. McCarley (1959) presented data on 84 specimens of 8 species from 12 counties but recent changes in the taxonomy of bats make the paper outdated and difficult to use. Davis (1974) mapped county locality records, gave a recent taxonomic arrangement of the species, and summarized much of the natural history information for bats in the area. His book does not give exact collecting localities within counties, institutions where specimens are deposited, or literature references.

In an attempt to document all records of bats collected in East Texas, we have examined 749 specimens from 29 counties. Also, for the past 2 years, one of us (Weynand) studied the distribution of bats on a selected area in Newton County in extreme eastern Texas. Information is given herein for 12 species of bats, including collecting localities and the institutions where specimens are deposited. In addition, pertinent natural history information on bats from Newton County is presented.

## INTRODUCTION

### *Region Studied*

The region herein defined as "East Texas" is essentially the same as that delimited by McCarley (1959). The eastern and southern boundaries are the Texas-Louisiana state line and the Gulf of Mexico, respectively (Figure 1). The Brazos River bounds the southern half of the western limits of the area. The remaining boundary is a line running from the Brazos River in Robertson County northeastward to the Red River in Lamar County and thence to the Texas-Louisiana state line.

East Texas contains a portion of 2 biotic provinces—the western extremities of the Austroriparian and the eastern half of the Texan (Blair, 1950). The Austroriparian Province includes 4 of Tharp's (1926) vegetational regions: oak-hickory, long-leaf pine, coastal prairie, and pine-oak forest (Figure 2). Only 2 bats (*Pipistrellus subflavus* and *Lasiurus borealis*) were among the 47 species of mammals listed for the Austroriparian Province by Blair (1950). The portion of the Texas Province in East Texas included parts of Tharp's (1926) coastal prairie, oak-hickory, and pine-oak regions. Of the 49 species of mammals occurring in this province, 41 also occur in the Austroriparian (Blair, 1950). No bats are listed as members of the Texan fauna by Blair (1950).

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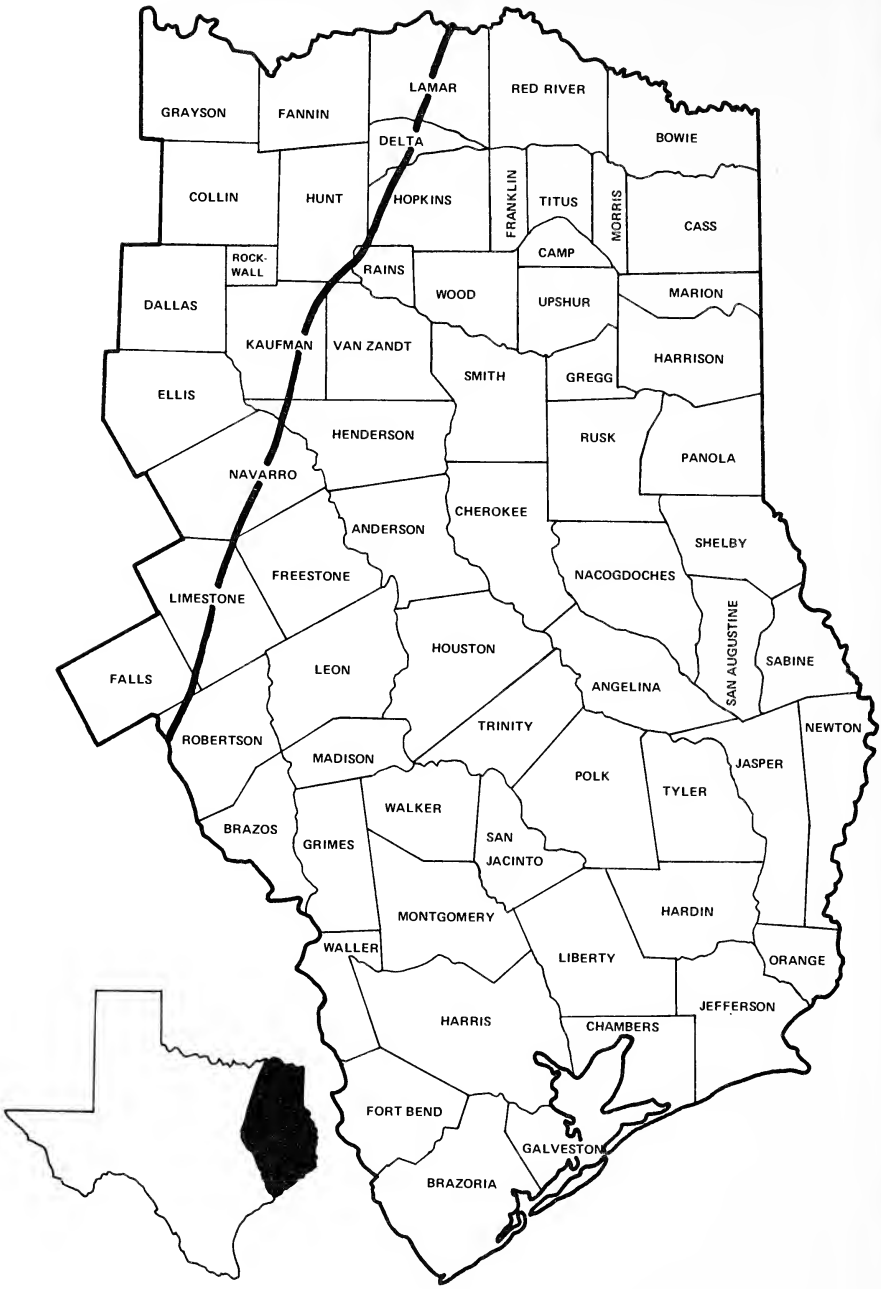


Figure 1. The East Texas region (dark area of the Texas map; counties included are to the right of the heavy line).

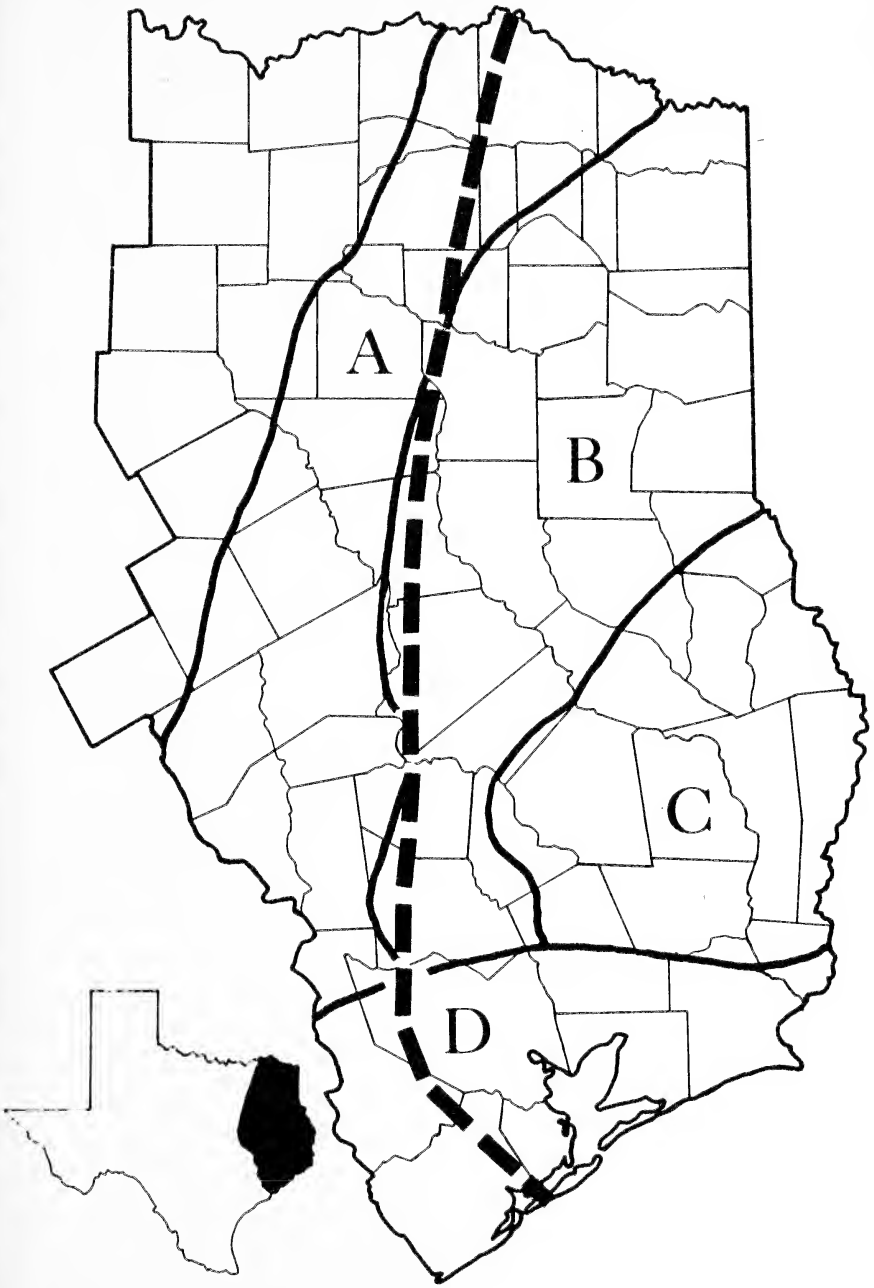


Figure 2. Map of East Texas illustrating biotic provinces and vegetational regions within the area. The heavy broken line separates the Texan (left of the line) from the Austroriparian (right of the line) Biotic Province. Solid lines separate the 4 vegetational regions: (A) oak-hickory; (B) pine-oak forest; (C) long-leaf pine; and (D) coastal prairie.

Newton County is situated in extreme eastern Texas on the Texas-Louisiana border (Figure 1). The mammal fauna on the proposed site of a nuclear power plant near Burkeville was inventoried during 1972-1974 (Inglis, *et al.*, 1974). Using conventional collecting techniques (including use of guns and mist nets), bats were sampled on the power plant site in and over essentially 4 distinct situations: streams in bottomlands, permanent man-made ponds in forested areas, flyways in forested areas, and man-made structures such as old wells, cisterns, and abandoned buildings.

*Accounts of Species*

Specimens examined in the account below are deposited in the following collections (museum abbreviations given in parentheses): Texas Cooperative Wildlife Collection, Texas A&M University (TCWC); Stephen F. Austin State University Vertebrate Collection (SFAVC); University of Texas at Arlington Collection of Vertebrates (UTAVC); The Museum, Texas Tech University (TTU); Museum of Zoology, Louisiana State University (LSUMZ); Bird and Mammal Laboratories, U.S. Bureau of Sport Fisheries and Wildlife (BS); The University of Kansas, Museum of Natural History (KU); and University of California, Museum of Vertebrate Zoology, Berkeley (MVZ). The use of common names and species accounts follow the sequence of species given in Jones, *et al.*, (1975). Counties are listed from west to east and north to south. Unless otherwise indicated, "additional records" are from the literature.

*Myotis lucifugus* (Le Conte). Little Brown Bat.

This species has not been previously recorded from East Texas. The only other record from Texas is that of a single specimen collected prior to 1900 at Fort Hancock in Hudspeth County. This specimen was referred to the subspecies *M. l. occultus* by Findley and Jones (1967). A Newton County specimen is clearly referable to *M. l. lucifugus* on both geographical and morphological grounds. This specimen was taken along Mill Creek under ecological conditions described below for *M. austroriparius*. The nearest locality from which this subspecies has been recorded is in central Arkansas, approximately 200 mi northeast of Newton County (Sealander and Young, 1955).

Specimens examined: total, 1. Newton Co.: 12 mi N Burkeville (TCWC).

*Myotis austroriparius* (Rhoads). Southeastern Myotis.

The Southeastern Myotis, which ranges from the Ohio River Valley into the southeastern states, reaches its western limits in extreme eastern Texas. Although this species is abundant in Louisiana (Lowery, 1974), previous collecting records suggest it is rare in East Texas. Packard (1966) reported the first Texas specimen from Bowie County; Michael, *et al.*, (1970) reported a single specimen from Panola County. We follow LaVal (1970) in considering *M. austroriparius* as monotypic; thus, no subspecific assignment has been made for Texas specimens.

The frequency with which this bat was taken in Newton County suggests it may be more common in East Texas than previously suspected. Specimens were obtained from Mill and Indian creeks, 12 and 8.5 mi N Burkeville, respectively. Both creeks are slow-moving narrow waterways surrounded by buttonbush and blackberry bushes. The adjacent woods consist of large upland areas of loblolly and shortleaf pines, narrow beach magnolia bottoms, and stands of hardwood trees (white oak, red oak, and hickory). The crown closure above the creeks ranged from 50 to 100% and the trees ranged in height from 50 to 100 ft.

Specimens examined: total, 9. Panola Co.: 8 mi SW Gary, 1 (TCWC). Newton Co.: 12 mi N Burkeville, 5 (TCWC): 8.5 mi N Burkeville, 2 (TCWC). Bowie Co.: New Boston, 1 (TTU).

*Lasionycteris noctivagans* (Le Conte). Silver-Haired Bat.

The only record of the silver-haired bat in East Texas is that of a female found alive hanging from a fence in a residential section of Galveston on 24 September 1975 (Martin, *In Press*). The nearest place in Texas where this species has been recorded is from Bandera County which is over 300 airline mi west of Galveston (Blair, 1952). Since this species' migratory habits are well-known, the Galveston specimen probably represents a migrant. Silver-haired bats have been observed migrating at sea along the Main coast (Lowery, 1974).

Specimens examined: total, 1. Galveston Co.: Galveston (TCWC).

*Pipistrellus subflavus* (F. Cuvier). Eastern Pipistrelle.

This species has been recorded from 10 East Texas counties. It has been collected in every month of the year and is apparently a permanent resident of the area. In Newton County, it was commonly collected along streams in bottomlands and in flyways in forests. Specimens from East Texas are referable to the subspecies *P. s. subflavus* on both morphological and geographical grounds.

Specimens examined: total, 42. Rusk Co.: 4 mi SE Gary, 4 (SFAVC). Anderson Co.: Long Lake, 3 (BS). Panola Co.: Lake Murvaul, 1 (SFAVC). Shelby Co.: 15 mi N Center, 1 (TTU); 14 mi N Center, 4 (SFAVC); cave between Timpson and Gary, 1 (SFAVC); no specific locality, 6 (LSUMZ). Nacogdoches Co.: cave 4 mi SW Garrison, 7 (SFAVC); 15 mi E Nacogdoches-Garrison Hwy, 1 (SFAVC); 1 mi S Cushing, 1 (SFAVC); Hwy 204, 10 mi E Cushing, 1 (SFAVC); Nacogdoches, 1 (SFAVC). Walker Co.: 11 mi NW Waverly, 1 (TMM). Polk Co.: 4 mi E Livingston, 2 (TCWC); 3 mi S Livingston, 1 (TCWC). Newton Co.: 9.3 mi N Burkeville, 1 (TCWC); 12 mi N Burkeville, 2 (TCWC); 11.5 mi N Burkeville, 1 (TCWC); 8.5 mi N Burkeville, 1 (TCWC). Harris Co.: Huffman, 1 (TCWC). Galveston Co.: Clear Creek, 1 (BS).

*Eptesicus fuscus* (Palisot de Beauvois). Big Brown Bat.

The big brown bat has been recorded from only 7 counties in East Texas and all recorded specimens are referable to the subspecies *E. f. fuscus*. All collecting records are within the pine-oak forest and long-leaf pine vegetational regions.

Available evidence suggests the favored roosts of this species in East Texas are man-made structures. In July 1975, we located a colony of approximately 35 big brown bats in a crack between a brick chimney and an old house within the city limits of Gilmer, Upshur County. Specimens have been collected from East Texas in all months except November and December.

Specimens examined: total, 27. Upshur Co.: Gilmer, 13 (TCWC). Marion Co.: Jefferson, 1 (BS). Nacogdoches Co.: SFA Campus, 4 (SFAVC); 1 (TTU); Nacogdoches, 1 (SFAVC). Trinity Co.: Trinity, 1 (TCWC). Walker Co.: 2 mi SW Huntsville, 2 (TCWC). San Jacinto Co.: 5 mi NW Cleveland, 2 (TTU). Hardin Co.: Sour Lake, 1 (BS); Grady, 1 (BS).

*Lasiurus borealis* (Müller). Red Bat.

This species is one of the most common bats in East Texas. It has been recorded from 23 counties and in all major vegetation regions. In Newton County, red bats were commonly collected along streams in bottomlands, in flyways in forests, and around man-made ponds. They have been collected at all seasons of the year and appear to be permanent residents of the area. East Texas specimens are referable to the subspecies *L. b. borealis*.

Specimens examined: total, 137. Lamar Co.: Arthur City, 3 (BS); Paris, 3 (BS). Red River Co.: Clarksville, 1 (BS). Bowie Co.: 8 mi N New Boston, 1 (TCWC). Van Zandt Co.: 1 mi SW Grand Saline, 1 (TTU). Smith Co.: Tyler, 1 (TCWC). Rusk Co.: 1.6 mi NE New London, 2 (SFAVC). Marion Co.: Jefferson, 2 (BS). Panola Co.: 6 mi E Mt. Enterprise, 1 (SFAVC); 7.5 mi ENE Carthage, 4 (TCWC). Cherokee Co.: Maydelle, 1 (SFAVC); 3 mi W Forrest, 1 (SFAVC), 1 (TTU). Shelby Co.: Choice, 1 (LSUMZ). Nacogdoches Co.: Martinsville, 1 (SFAVC); SFA Campus, 5 (SFAVC); SFA Experimental Forest, 2 (TTU); Sam Rayburn Reservoir, 1 (SFAVC); Nacogdoches, 1 (SFAVC), 5 (TTU). Angelina Co.: 23 mi S Nacogdoches, 1 (TTU); 1 mi S Lufkin, 1 (SFAVC); no specific locality, 1 (SFAVC). Trinity Co.: 1.3 mi E Trinity, 1 (TCWC); Trinity, 1 (TCWC). Walker Co.: 16 mi SW Huntsville, 1 (TCWC); 2 mi NE Huntsville, 2 (TCWC); Huntsville, 1 (TCWC). San Jacinto Co.: San Jacinto River, Farm Rd 995, 8 (TTU); 5 mi NW Cleveland, 13 (TTU); San Jacinto River, Farm Rd 945, 13 (TTU). Jasper Co.: Bouton Lake, 1 (UTAVC). Newton Co.: 7.5 mi N Burkeville, 5 (TCWC); 11.5 mi N Burkeville, 2 (TCWC), 7 mi N Burkeville, 1 (TCWC); Newton, 1 (TTU). Hardin Co.: Sour Lake, 1 (BS). Brazos Co.: Bryan, 3 (TCWC); College Station, 11 (TCWC); 6.5 mi SE College Station, 4 (TCWC); 6.5 mi SW College Station, 1 (TCWC). Montgomery Co.: 20 mi SW Huntsville, 4 (TMM). Liberty Co.: 12 mi N Dayton, 3 (TTU); 20 mi NW Liberty, 1 (BS). Harris Co.: Houston, 4 (TCWC); no specific locality, 1 (SFAVC). Jefferson Co.: Port Arthur, 6 (SFAVC), 6 (TTU). Galveston Co.: Bolivar Peninsula, 7.5 mi NE Ferry landing, 1 (TMM).

*Lasiurus seminolus* (Rhoads). Seminole Bat.

The seminole bat is abundant throughout the pine-oak and long-leaf pine forest regions of East Texas; it is less common in the oak-hickory region. Since it has been taken in East Texas from February through November, it probably

is a year-long resident in the area. In Newton County, this bat was collected along Mill and Indian creeks under ecological conditions as described in the account of *M. austroriparius.* This species is monotypic.

Specimens examined: total, 71. Panola Co.: 6 mi E Mt. Enterprise, 1 (SFAVC); 7.5 mi ENE Carthage, 1 (TCWC). Cherokee Co.: 3 mi W Forrest, 1 (SFAVC). Shelby Co.: 4 mi S Joaquin, 1 (SFAVC); 2.3 mi SE Patroon, 1 (SFAVC). Nacogdoches Co.: 9.5 mi SW Nacogdoches, 1 (TMM); Nacogdoches, 6 (SFAVC); SFA Campus, 2 (SFAVC), 1 (TTU). Trinity Co.: Trinity, 1 (TCWC). Sabine Co.: 5 mi N Geneva, 1 (TCWC); no specific locality, 1 (SFAVC). San Jacinto Co.: 5 mi NW Cleveland, 15 (TTU); Farm Rd 945 San Jacinto River, 10 (TTU). Polk Co.: 3 mi S Livingston, 1 (TCWC); 4 mi E Livingston, 1 (TCWC); no specific locality, 2 (SFAVC). Newton Co.: 8.5 mi N Burkeville, 1 (TCWC); 11.5 mi N Burkeville, 6 (TCWC). Montgomery Co.: 5 mi S Richards, 1 (TCWC). Harris Co.: Houston, 10 (LSUMZ); no specific locality, 4 (TCWC). Liberty Co.: 12 mi N Dayton, 1 (TTU). Jefferson Co.: 11.7 mi W Sabine Pass, Hwy 87, 1 (TCWC).

Additional records: Rusk Co. (McCarley, 1959); Harris Co. (Olsen, 1966).

*Lasiurus cinereus* (Palisot de Beauvois). Hoary Bat

This species has not been reported previously from East Texas, although it is relatively common in other parts of Texas and throughout Louisiana. The East Texas specimen, which is referable to the subspecies *L. c. cinereus,* was taken near Cleveland in San Jacinto County on 13 January 1974. The nearest recorded Texas specimen is from McLennan County, 140 miles northwest of Cleveland.

Specimens examined: total, 1. San Jacinto Co.: 5 mi NW Cleveland, 1 (TTU).

*Lasiurus intermedius* (H. Allen). Northern Yellow Bat.

Collecting records indicate this species is one of the rarest of the tree bats in East Texas. Until recently, it was known only from Harris County. However, on 24 April 1975, one of us (Wilkins) netted an adult female over a narrow pool of water in Brazos County. The uterus of this female contained four embryos which were each 5 mm in crown-rump length. According to Hall and Jones (1961), East Texas specimens are referable to the subspecies *L. i. floridanus* (Miller) rather than to *L. i. intermedius* (H. Allen) which occurs in South Texas.

Specimens examined: total, 8. Brazos Co.: 8 mi SW College Station, 1 (TCWC). Harris Co.: Houston, 1 (KU), 2 (MVZ), 2 (TCWC); 4 mi N Huffman, 1 (TCWC). Fort Bend Co.: Brawner Ranch, 2 mi S Dewalt, 1 (TTU).

*Nycticeius humeralis* (Rafinesque). Evening Bat.

This species is widely distributed throughout East Texas and is probably the most common bat in the area. It occurs in all major vegetation regions and is represented in collections by more than 150 specimens collected from 16 counties. In Newton County we collected specimens along streams in bottomlands and around man-made ponds. In Nacogdoches County, evening bats have been collected

in all months of the year, although records indicate they are more abundant in summer and fall than in the winter (Table 1). Specimens from East Texas are referable to the subspecies *N. h. humeralis* on both geographical and morphological grounds.

TABLE 1

Monthly Collecting Records of *Nycticeius humeralis* from Nacogdoches County, Texas.

| Month | Males | Females | Sex Unknown* | Totals |
|---|---|---|---|---|
| J | 1 | 0 | 1 | 2 |
| F | 2 | 0 | 1 | 3 |
| M | 0 | 4 | 0 | 4 |
| A | 1 | 1 | 3 | 5 |
| M | 0 | 1 | 0 | 1 |
| J | 1 | 3 | 5 | 9 |
| J | 1 | 9 | 9 | 19 |
| A | 4 | 3 | 0 | 7 |
| S | 6 | 4 | 1 | 11 |
| O | 6 | 5 | 2 | 13 |
| N | 5 | 2 | 1 | 8 |
| D | 4 | 0 | 0 | 4 |
| TOTALS | 31 | 32 | 23 | 86 |

*Sex not recorded on specimen labels.

Non-geographic variation has not been previously investigated in *Nycticeius humeralis*. We utilized the large sample of evening bats from Nacogdoches County to determine the extent of individual and sexual variation in this species. Of the 10 external and cranial characters we examined, all but 2 (interorbital constriction and width across the upper molars) exhibited significant secondary sexual dimorphism (see Table 2). In all characters, females averaged larger than males. Coefficients of variation (CV's) of external measurements averaged larger than those of cranial features. Of the cranial measurements, length of palate had the highest CV. With the exception of cranial breadth and width across the upper molars, CV's of males averaged larger than those of females.

Specimens examined: total, 160. Lamar Co.: Paris, 3 (BS); Arthur City, 1 (BS). Bowie Co.: 8 mi N New Boston, 1 (TCWC); Texarkana, 3 (BS). Marion Co.: Jefferson, 1 (BS). Rusk Co.: 1.6 mi NE New London, 6 (SFAVC). Anderson Co.: Palestine, Gus Engling Wildlife Management Area, 1 (TCWC). Cherokee Co.: Bates Farm, 3 mi W Forrest, 3 (SFAVC); Cherokee Co., 1 (SFAVC). Nacogdoches Co.: Nacogdoches, 33 (TTU), 56 (SFAVC); SFA Campus, 12 (SFAVC); 1/2 mi E Nacogdoches, 1 (SFAVC); 1 mi E Nacogdoches, 1 (SFAVC); 11 mi SW Nacogdoches, 1 (TTU).

TABLE 2

Statistical Data for a Population of *Nycticeius humeralis* (Males and Females) from Nacogdoches County, Texas. F-values Marked with an Asterisk Significant at $P \leq 0.05$.

| | MALES | | | | FEMALES | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Character | N | Mean | SD | CV | N | Mean | SD | CV | F-value |
| Forearm length | 19 | 35.20 | 1.35 | 3.83 | 36 | 36.18 | 0.98 | 2.72 | 9.50* |
| Length 3rd metacarpal | 26 | 32.83 | 1.42 | 4.32 | 44 | 34.04 | 1.19 | 3.51 | 14.54* |
| 3rd phalanx, digit 3 | 26 | 11.78 | 0.66 | 5.61 | 43 | 12.47 | 0.60 | 4.81 | 20.27* |
| Skull length | 20 | 14.25 | 0.40 | 2.84 | 18 | 14.55 | 0.33 | 2.28 | 6.38* |
| Zygomatic breadth | 17 | 9.78 | 0.28 | 2.85 | 25 | 10.01 | 0.26 | 2.57 | 7.72* |
| Greatest width across braincase | 21 | 8.09 | 0.18 | 2.30 | 19 | 8.31 | 0.23 | 2.83 | 11.12* |
| Interorbital constriction | 24 | 3.90 | 0.15 | 3.92 | 36 | 3.90 | 0.12 | 3.23 | 0.03 |
| Length maxillary toothrow | 24 | 5.04 | 0.22 | 4.31 | 35 | 5.19 | 0.22 | 4.22 | 7.01* |
| Width across upper molars | 24 | 6.34 | 0.15 | 2.33 | 36 | 6.43 | 0.17 | 2.59 | 3.75 |
| Length of palate to front of incisors | 15 | 5.70 | 0.35 | 6.17 | 27 | 6.07 | 0.41 | 6.72 | 8.66* |

Shelby Co.: Shelbyville, 2 (SFAVC). Sabine Co.: Pineland, 1(TCWC). Trinity Co.: Trinity, 5 (TCWC). San Jacinto Co.: 5 mi NW Cleveland, 7 (TTU); Jct Farm Rd 945, San Jacinto River, 1 (TTU). Jasper Co.: Jasper, 3 (BS). Newton Co.: 12 mi N Burkeville, 1 (TCWC); 11.5 mi N Burkeville, 2 (TCWC); 9.2 mi N Burkeville, 3 (TCWC); Brazos Co.: 3 mi S Bryan, 1 (TCWC); 2 mi SE Bryan, 1 (TCWC); College Station,

5 (TCWC); 8 mi SE College Station, 1 (TCWC). Montgomery Co.: 5 mi S Richards, 1 (TCWC). Harris Co.: Houston, 1 (BS). Fort Bend Co.: Brawner Ranch, 3 mi S Dewalt, 1 (LSUMZ).

Additional records, Harris Co.: Houston (Olsen, 1966).

*Plecotus rafinesquii* (Lesson). Rafinesque's Big-eared Bat.

This is another species that reaches the western limits of its range in East Texas. Michael and Birch (1967) recorded the first Texas specimens from Polk County. This bat was relatively common in Newton County where it seemed to prefer wells and cisterns as roosting sites. Of the 5 bats collected there, 4 were taken in wells and cisterns; 1 was obtained in a mist net strung among junipers and loblolly pines. Specimens were obtained in November, December, and June. Our observations support the conclusions of Jones and Suttkus (1975) that *P. rafinesquii* is found in small numbers at scattered localities. This species is monotypic.

Specimens examined: total, 36. Marion Co.: 27 mi W Jefferson, 1 (SFAVC). Nacogdoches Co.: Nacogdoches, 14 (SFAVC); 10 mi SE Nacogdoches, 5 (TCWC). Sabine Co.: 3 mi NE Milam, 3 (SFAVC). Polk Co.: 1 mi S Neches River, 3 mi E Hwy 59, 2 (TCWC); no specific locality, 5 (SFAVC). Newton Co.: 6 mi N Burkeville, 1 (TCWC); 7 mi N Burkeville, 1 (TCWC); 10 mi N Burkeville, 2 (TCWC); 9.5 mi N Burkeville, 1 (TCWC). Hardin Co.: 7 mi SE Silsbee, 1 (TCWC).

*Tadarida brasiliensis* (Saussure). Brazilian Free-tailed Bat.

Two kinds of Brazilian Free-tailed Bats have been reported from the southern United States and both are known from East Texas. *T. b. mexicana* (Saussure) is a migratory, cave-dwelling (sometimes house-dwelling) bat of the southwestern United States which reaches the western limits of its range in East Texas. These bats do not hibernate, and during June and July the females give birth to their young in caves where females congregate in extremely large numbers. The other form, *T. b. cynocephala* (LeConte), is a sedentary, tree- or house-dwelling bat of the southeastern United States which reaches the western limits of its range in East Texas. This bat hibernates during the winter and gives birth to its young during June and July.

The taxonomic status of *T. b. mexicana* and *T. b. cynocephala* has been debated for years. Carter (1962) and Davis (1974) consider them as distinct species, but certain recent authors (for example, Barbour and Davis, 1969; Lowery, 1974) have preferred to consider them both as subspecies of *T. brasiliensis* as was proposed by Schwartz (1955).

The primary distinctions between *cynocephala* and *mexicana* are ethological, although some morphological dissimilarity is evident between the two (Carter, 1962). Carter (1962) has suggested that *cynocephala* and *mexicana* are seasonally sympatric in East Texas because during early March, when both forms are presumably copulating, some *mexicana* pass through the western edge of the geographic range of *cynocephala*. If intergradation is possible, it should occur in an

area between the forested region of East Texas and the Balcones Escarpment, and the resulting populations should be morphologically and ethologically intermediate between *T. cynocephala* and *T. mexicana* (Carter, 1962).

In order to allocate and define populations of free-tailed bats inhabiting East Texas, we found it necessary to study geographic variation among populations from Texas and the southeastern United States. A total of 359 specimens was examined from the following places (sample sizes given in parenthesis): 1. Florida (21 females); 2. Georgia (14 males, 29 females); 3. Alabama (6 males; 18 females); 4. Sabine Co., Texas (58 males, 51 females); 5. Nacogdoches Co., Texas (14 males, 10 females); 6. Anderson Co., Texas (10 males); 7. Harris Co., Texas (6 males, 15 females); 8. Grimes Co., Texas (5 males, 5 females); 9. Brazos Co., Texas (10 males, 8 females); 10. Kerr Co., Texas (16 females); 11. Comal Co., Texas (13 males, 23 females); 12. Val Verde Co., Texas (16 males, 7 females). For each specimen, we recorded (to the nearest 0.01 mm using dial calipers) the following cranial measurements (numbers refer to characters in Table 2): (1) greatest length of skull, (2) zygomatic breadth; (3) greatest width across the braincase; (4) interorbital constriction; (5) length of maxillary toothrow ($C\text{-}M^3$); (6) width across molars ($M^3\text{-}M^3$); (7) length of palate measured from front of incisors to posteriormost point near midline of palate; (8) greatest length of mandible; and (9) length of mandibular toothrow ($C\text{-}M_3$).

Since earlier studies have shown that significant sexual dimorphism exists in populations of *T. brasiliensis,* we treated the sexes separately in analyzing geographic variation. To assess the degree of divergence among samples, we used a multivariate analysis of variance (MANOVA) and a canonical analysis program in the Statistical Analysis System (SAS) designed and implemented by Barr and Goodnight (Service, 1972). Canonical analysis of the data aimed at providing weighted combinations of the measurements which maximize the distinction between the groups (for a detailed discussion of the methodology, see Schmidly and Hendricks, *In Press*). Sample means and individual specimens were plotted on those canonical variates that account for the greatest fractions of total variation. The relative importance of each original variable to a particular canonical variate was computed by multiplying the vector variable coefficient by the median value of the dependent variable, summing all variable values for a particular vector, and then computing the percent of relative importance of each variable per vector.

The first 3 canonical variates were computed from the variance-covariance matrix among the 9 cranial characters for all samples of males and females separately. For males, the first canonical variate expresses 60.6% of the phenetic variation; the second, 14.9; and the third, 10.1. These same values for females are 59.2, 22.8 and 7.1%, respectively. Two-dimensional plots of the first 2 canonical variates (including the mean and 2 standard errors either side of the mean for each sample) for males and females are shown in Figure 3.

Examination of Figure 3 (males) reveals that the primary separation of samples currently defined as *cynocephala* and *mexicana* occurs along Vector I. Samples of *cynocephala* (2, 3, 4, 5, and 7) cluster on Side A of the graph and include 3

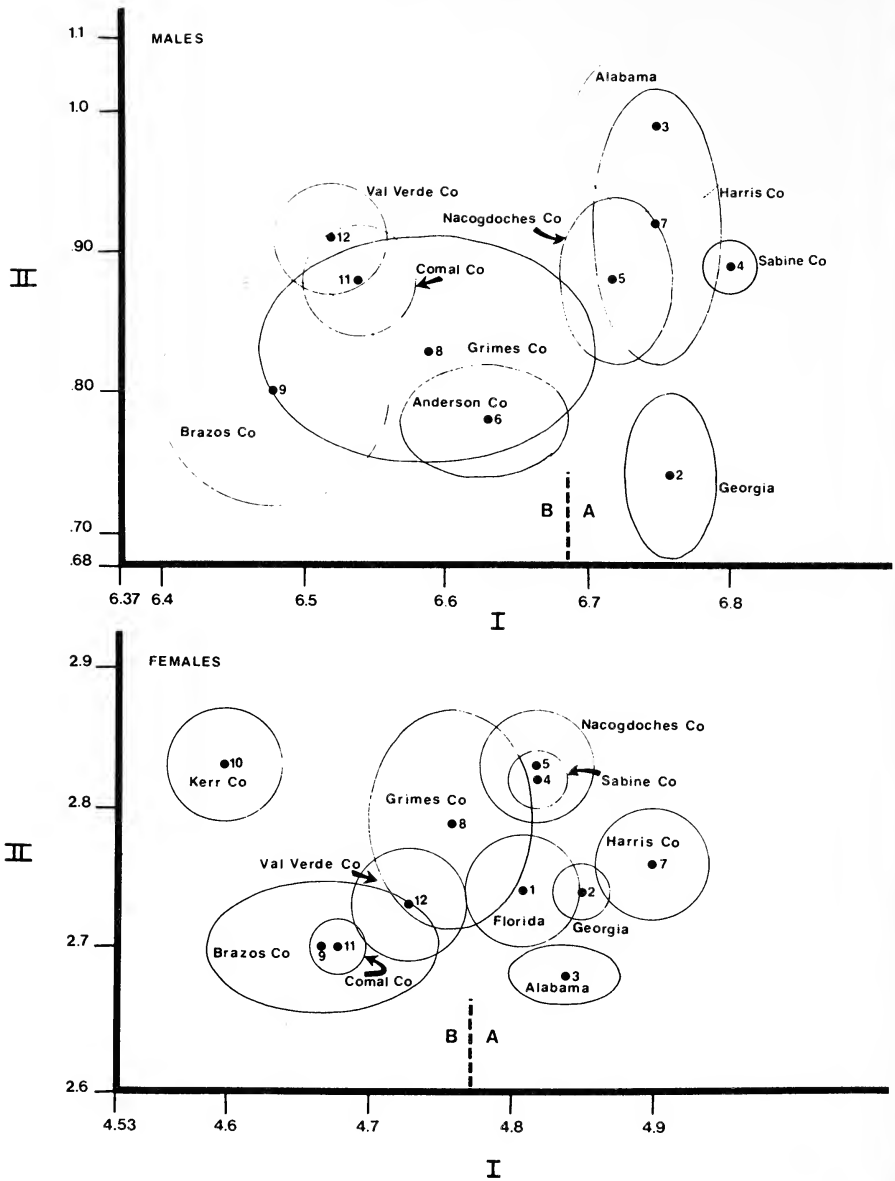


Figure 3. Projections of the first 2 canonical vectors illustrating the phenetic position of male (top) and female (bottom) samples of *Tadarida brasiliensis*. Numbers are positioned at the mean value for each sample in the character space; the ellipse surrounding each number represents 2 standard errors around the mean. See text for key to samples.

samples (4, 5, and 7) from Sabine, Nacogdoches, and Harris Counties in East Texas. Samples of *mexicana* (6, 9, 11, and 12) cluster on Side B of the graph and include two samples (6 and 9) from Brazos and Anderson Counties in East Texas. Sample 8 from

Grimes County in East Texas is intermediate and overlaps both the *cynocephala* and *mexicana* clusters. Those characters which have the greatest influence on Vector I and hence which contribute most to the separation of *cynocephala* and *mexicana* are greatest length of the skull, zygomatic breadth, and breadth of the cranium (Table 3). In all of these features, specimens of *cynocephala* average larger than specimens of *mexicana* (see Table 4).

TABLE 3

Variable Coefficients for Canonical Variates I and II with an Estimate of the % Influence of Each Variable on Each Vector for Samples of Male and Female *Tadarida brasiliensis.*

| | MALES | | | |
|---|---|---|---|---|
| | Vector I | | Vector II | |
| Character | Variable Coefficient | Percent Influence | Variable Coefficient | Percent Influence |
| 1 | 0.162 | 31.49 | -0.026 | 4.32 |
| 2 | 0.227 | 25.76 | -0.007 | 0.68 |
| 3 | 0.134 | 14.24 | -0.091 | 8.29 |
| 4 | 0.167 | 7.52 | 0.221 | 8.51 |
| 5 | 0.008 | 0.55 | -0.096 | 5.63 |
| 6 | -0.083 | 6.50 | -0.022 | 1.48 |
| 7 | 0.038 | 2.49 | -0.183 | 10.25 |
| 8 | 0.043 | 5.91 | 0.388 | 45.71 |
| 9 | 0.047 | 5.53 | -0.236 | 15.11 |
| | | FEMALES | | |
| 1 | 0.067 | 15.90 | 0.034 | 14.56 |
| 2 | 0.211 | 29.38 | 0.008 | 2.02 |
| 3 | 0.102 | 13.37 | -0.007 | 1.66 |
| 4 | 0.101 | 10.10 | 0.185 | 18.45 |
| 5 | 0.026 | 2.60 | 0.077 | 11.74 |
| 6 | 0.036 | 3.49 | -0.019 | 3.33 |
| 7 | -0.129 | 12.90 | 0.191 | 27.38 |
| 8 | -0.038 | 3.80 | 0.035 | 10.60 |
| 9 | 0.147 | 13.46 | -0.062 | 10.25 |

Examination of Figure 3 (females) reveals the same general pattern as that observed for males. Again the major separation of *cynocephala* and *mexicana* is along Vector I. Samples of *cynocephala* (1, 2, 3, 4, 5, and 7) congregate on Side A of the graph and samples of *mexicana* (9, 10, 11, and 12) on Side B. Once again Sample 8 from Grimes County in East Texas is intermediate and overlaps both the *cynocephala* and *mexicana* clusters. Characters which have the highest percent

TABLE 4

Selected Cranial Measurements (Mean ± 1 Standard Deviation) of Samples of *T. brasiliensis* from the Southeastern United States.

| Sex | N | Greatest length of skull | Zygomatic breadth | Greatest width across braincase | Length of mandibular toothrow |
|---|---|---|---|---|---|
| | | | Georgia (*cynocephala*) | | |
| Male | 14 | 17.42 ± 0.17 | 10.19 ± 0.14 | 9.61 ± 0.12 | 6.67 ± 0.16 |
| Female | 29 | 17.02 ± 0.28 | 10.08 ± 0.17 | 9.46 ± 0.16 | 6.61 ± 0.18 |
| | | | Sabine Co., Texas (*cynocephala*) | | |
| Male | 58 | 17.50 ± 0.23 | 10.30 ± 0.18 | 9.60 ± 0.20 | 6.78 ± 0.20 |
| Female | 51 | 17.10 ± 0.25 | 10.06 ± 0.15 | 9.49 ± 0.20 | 6.57 ± 0.23 |
| | | | Grimes Co., Texas (*intergrades*) | | |
| Male | 5 | 17.29 ± 0.24 | 9.85 ± 0.40 | 9.30 ± 0.22 | 6.60 ± 0.28 |
| Female | 5 | 17.06 ± 0.11 | 10.00 ± 0.18 | 9.30 ± 0.12 | 6.50 ± 0.12 |
| | | | Brazos Co., Texas (*mexicana*) | | |
| Male | 10 | 16.65 ± 0.07 | 9.88 ± 0.11 | 9.05 ± 0.07 | 6.50 ± 0.07 |
| Female | 8 | 16.71 ± 0.20 | 9.74 ± 0.19 | 9.15 ± 0.20 | 6.32 ± 0.12 |
| | | | Comal Co., Texas (*mexicana*) | | |
| Male | 13 | 17.01 ± 0.25 | 9.89 ± 0.26 | 9.23 ± 0.15 | 6.45 ± 0.17 |
| Female | 23 | 16.69 ± 0.26 | 9.74 ± 0.18 | 9.20 ± 0.12 | 6.38 ± 0.21 |

influence on Vector I for females include zygomatic breadth, greatest length of skull, length of the lower mandibular toothrow, and breadth of cranium (Table 3). In all of these features, specimens of *cynocephala* average larger than specimens of *mexicana* (Table 4).

We interpret the morphological evidence as indicating that although samples of *cynocephala* and *mexicana* are somewhat morphologically distinct, there is evidence of morphological intergradation where their ranges come in contact in East Texas. The sample from Navasota in Grimes County is morphologically intermediate and overlaps both the sample of *mexicana* from College Station in Brazos County and samples of *cynocephala* from eastern Texas. On the basis of the morphological analysis, we find it difficult to justify recognizing *cynocephala* and *mexicana* as separate species. However, this interpretation does not eliminate the possibility that ethological isolation, which would completely prevent them from interbreeding, might exist between the two forms. Supposedly, copulation in the migratory *mexicana* occurs in Mexico, whereas copulation in the nonmigratory *cynocephala* takes place within its range in the United States (Barbour and Davis,

1969). Spenrath and LaVal (1974) studied the population dynamics of the colony of *T. b. mexicana* at College Station and found it to be somewhat atypical in comparison to other colonies of this bat in that males remain in the colony throughout the year. We have studied this colony since 1971 and our data support Spenrath and LaVal's (1974) conclusions. The year-round presence of males of *T. b. mexicana* at College Station, a locality only 100 mi from colonies of *T. b. cynocephala* in eastern Texas, plus the fact that a population of bats morphologically intermediate between *mexicana* and *cynocephala* is located at Navasota in Grimes County strongly suggests that gene flow exists between populations of these 2 taxa. Obviously the behavior of the bats in Grimes County needs to be investigated to support or refute our hypothesis. But until these studies are conducted, we feel the data now available best support the conclusion that *cynocephala* and *mexicana* are conspecific. We follow Schwartz (1955) in treating the 2 taxa as subspecies of the wide-ranging species *T. brasiliensis*.

Specimens examined: total, 256. *T. b. cynocephala.* Cherokee Co.: 1 (SFAVC). Shelby Co.: 20 mi S Center, 1 (SFAVC). Nacogdoches Co.: Nacogdoches, 59 (SFAVC), 13 (TTU); 11 mi SW Nacogdoches, 1 (TTU). Angelina Co.: Lufkin, 2 (SFAVC). Sabine Co.: Pineland, 107 (TCWC); 11 mi N Hemphill, 1 (SFAVC). Harris Co.: Houston, 20 (TCWC). *T. b. mexicana.* Anderson Co.: Palestine, 12 (TCWC). Brazos Co.: College Station, 17 (TCWC). Grimes Co.: Navasota, 22 (TCWC).

*Distributional Patterns*

Distributional patterns of East Texas bats by biotic province and vegetational regions are presented in Table 5. Of the 2 biotic provinces in East Texas, the Austroriparian supports the most diverse chiropteran fauna. All 12 East Texas

TABLE 5

Distributions of East Texas Bats by Biotic Provinces and Vegetation Regions.

| Species | Biotic Province | | Vegetational Region | | | |
|---|---|---|---|---|---|---|
| | Austro-riparian | Texan | Oak-Hickory | Pine-Oak | Long-leaf Pine | Coastal Prairie |
| *Pipistrellus subflavus* | X | X | X | X | X | X |
| *Eptesicus fuscus* | X | | | X | X | X |
| *Lasiurus borealis* | X | X | X | X | X | X |
| *Lasiurus seminolus* | X | | | X | X | X |
| *Lasiurus cinereus* | X | | | X | | |
| *Lasiurus intermedius* | X | X | X | | | X |
| *Nycticeius humeralis* | X | X | X | X | X | X |
| *Myotis austroriparius* | X | | | X | X | |
| *Myotis lucifugus* | X | | | | X | |
| *Lasionycteris noctivagans* | X | | | | | X |
| *Plecotus rafinesquii* | X | | | X | X | |
| *Tadarida brasiliensis* | X | X | X | X | X | X |
| TOTALS | 12 | 5 | 5 | 9 | 9 | 8 |

bats occur in this province whereas only 5 occur in the Texan Province. Seven species occur only in the Austroriparian Province, whereas none are restricted to the Texan Province. Among the 4 vegetational regions in East Texas, the western-most (oak-hickory) region supports the fewest species (5), whereas the pine-oak and long-leaf pine regions (each with 9 species) support the most diverse fauna. Eight species have been recorded from the coastal prairie region. Four species (*Pipistrellus subflavus, Lasiurus borealis, Nycticeius humeralis,* and *Tadarida brasiliensis*) are found throughout East Texas. Collecting records indicate that *Myotis lucifugus, Lasionycteris noctivagans,* and *Lasiurus cinereus* are rare in the area with only 1 county record for each.

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# A KEY TO THE LICE OF MAN AND DOMESTIC ANIMALS

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ABSTRACT

Dichotomous keys are given to the species of biting and sucking lice normally found on man and domestic animals. Illustrations of diagnostic characters used in the keys are included.

## INTRODUCTION

The following keys are designed primarily for the non-specialist to facilitate the identification of the lice parasitizing man and domestic animals. The terms used in most keys, particularly those unaccompanied by illustrations and often poorly defined, are difficult to interpret.

It is hoped that the keys will fulfill two basic needs: first, make the identification of the most frequently encountered species of lice as simple as possible by elimination of the seldom encountered species; and second, explain the more important terms used in the keys by the use of an adequate number of illustrations. Throughout the keys, I have avoided using characters of the genitalia to separate species. More often than not, a person will have a specimen of the opposite sex than that referred to in the key. Also, specimens must be in an exceptionally good condition and properly mounted so that the characters of the genitalia can be seen.

It is essential to remember that although the wording in a key is fixed, the organisms are not and some variation within species' populations may be encountered.

The keys will give an individual practice in identifying specimens of lice from domestic animals when the specific host is unknown. Generally, specimens can be identified simply by knowing the host, then confirmation of the determination by comparing the specimen with descriptions and illustrations in the laboratory is an easy matter. For the diagnosis of louse infestations, a host list, and photographs of several of the more common species found on domestic animals, the reader is referred to the works of La Page (1968) and Sloss (1973).

## KEY TO THE ORDERS OF LICE

1 Anterior margin of head acute, rostrum present, mouthparts adapted for piercing and sucking; opposing mandibles absent; tarsal claws single, often large . . . . . *Part I,* ANOPLURA

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1′ Anterior margin of head generally broadly rounded, rostrum absent, mouthparts adapted for chewing; opposing mandibles well developed, on ventral surface; tarsal claws small, either single or paired . . . . . *Part II,* MALLOPHAGA

## *Part I*

### KEY TO THE FAMILIES, GENERA AND SPECIES OF ANOPLURA OF DOMESTIC ANIMALS

1 Eyes well developed, with distinct lens (Figure 1) . . . . Pediculidae, 3

1′ Eyes vestigial or absent . . . . . 2

2(1′) Paratergal plates of abdominal segments heavily sclerotized forming lateral lobes (Figure 2); all legs of equal size (Figure 3) . . . Haematopinidae, 4

2′ Paratergal plates of abdominal segments absent; if present, greatly reduced and weakly sclerotized; first pair of legs smaller than second and third pairs (Figure 4) . . . . . Linognathidae, 6

#### *Family Pediculidae*

3(1) All legs equal in size and shape, tarsal claws slender (Figure 5); abdominal segments normal, with sclerotized paratergal plates, each plate bearing a spiracle (Figure 6) . . . (the head louse and body louse) *Pediculus humanus*

3′ First pair of legs reduced (Figure 5), second and third pairs greatly enlarged with robust tarsal claws (Figure 7); abdomen with 4 pairs of prominent lateral tubercles (Figure 8); a group of 3 distinct spiracles located dorsally on each side of the abdomen behind the third pair of legs . . . . . (the crab louse) *Pthirus pubis*

#### *Family Haematopinidae*

4(2) Head 2 to 3 times as long as wide (Figure 9) . . . . . 5

4′ Head about as long as wide (Figure 10); paratergal plates tuberculated (Figure 11) . . . (the shortnosed cattle louse) *Haematopinus eurysternus*

Another louse is recognized here, *H. quadripertusus,* the cattle tail louse. This louse is ecologically isolated from *H. eurysternus,* being found on the tail of cattle, but is morphologically identical with *H. eurysternus.*

5(4) Paratergal plates rounded (Figure 2); tarsal claws toothed (Figure 12) . . . . . (the hog louse) *Haematopinus suis*

5′ Paratergal plates tuberculate (Figure 11); tarsal claws simple, not toothed (Figure 13) . . . . (the horse sucking louse) *Haematopinus asini*

#### *Family Linognathidae*

6(2′) Abdominal segments with only one transverse row of setae; abdominal spiracles borne on small tubercles (Figure 14) . . . . . (the capillate cattle louse) *Solenopotes capillatus*

6′ Abdominal segments with more than one transverse row of setae; abdominal spiracles not borne on tubercles (Figure 15) . . . . . 7

7(6′) Postantennal region laterally produced (Figure 16) . . . . . (on sheep and goats) *Linognathus africanus*

7′ Postantennal region not laterally produced . . . . . 8

8(7′) Head twice as long as broad; preantennal region as long as broad (Figures 17, 18) . . . . . 9

8′ Head about as long as broad or slightly longer; preantennal region much broader than long (Figures 19, 20) . . . . . 10

9(8) Preantennal region elongate, apically acute (Figure 17a); lateral margins of postantennal regions straight, appearing rectangular (Figure 17b) . . . . . (the long-nosed cattle louse) *Linognathus vituli*

9′ Preantennal region acute (Figure 18a); lateral margins of postantennal regions slightly convex (Figure 18b) . (the goat sucking louse) *Linognathus stenopsis*

10(8′) Head as long as broad, preantennal region very short (Figure 19a), lateral margins of postantennal region slightly convex (Figure 19b) . . . . . (the sheep foot louse) *Linognathus pedalis*

10′ Head slightly longer than broad, preantennal region well developed, with lateral margins straight and apex blunt, (Figure 20a), lateral margins of postantennal region parallel (Figure 20b) . . . . . (the dog sucking louse) *Linognathus setosus*

## *Part II*

### KEY TO THE SUBORDERS, FAMILIES, GENERA AND SPECIES OF MALLOPHAGA OF DOMESTIC ANIMALS

1 Antennae distinctly clubbed or capitate, concealed in a groove on underside of head (Figure 21) . . . . . AMBLYCERA 2

1′ Antennae not clubbed or capitate, project from lateral margins of head (Figure 22) . . . . . ISCHNOCERA 6

### KEY TO THE FAMILIES OF THE SUBORDER AMBLYCERA

2(1) Underside of head with two long stout backward projecting spine-like processes; antennae clubbed (Figures 23, 24) . . . . . Boopidae

2′ Underside of head with spine-like processes reduced or absent; antennae not strongly clubbed (Figures 25, 26) . . . . . Menoponidae 3

#### *Family Boopidae*

There is one species of Boopidae, *Heterodoxus spininger,* found in the United States and occurs on dogs. *H. spininger* is easily recognized by the shape of the head and the larger backward projecting spines. The body is covered with large and numerous setae.

#### *Family Menoponidae*

3(2′) Underside of head with 2 short spine-like processes (Figure 25) . . . 4

3′ Underside of head without spine-like processes . . . . . (the shaft louse [on chickens]) *Menopon gallinae*

4(3) Abdominal tergites III-VII each with one transverse row of setae (Figure 27) . . . . . (on chickens) *Menacanthus pallidulus*

4′ Abdominal tergites III-VII each with two transverse rows of setae (Figure 28) . . . . . 5

5(4′) Dorsal portion of meso and metathorax with numerous short setae (Figure 29) . . . . . (the chicken body louse) *Menacanthus stramineus*

5′ Dorsal portion of meso and metathorax with a few short setae on the lateral margins (Figure 30) . . . . (on chickens) *Menacanthus cornutus*

## KEY TO THE FAMILIES OF THE SUBORDER ISCHNOCERA

6(1′) Tarsi each with two claws (Figure 31) . . . . . Philopteridae 7

6′ Tarsi each with one claw (Figure 32) . . . . . Trichodectidae 13

### *Family Philopteridae*

7(6) Temporal lobes angulate (Figures 33, 34) . . . . . 8

7′ Temporal lobes rounded (Figure 35) . . . . . 11

8(7) Temporal lobes extending backward and terminating in long stylet-like processes (Figure 36) . . (the large turkey louse) *Chelopistes meleagridis*

8′ Temporal lobes not produced into stylet-like processes . . . . . 9

9(8′) Lateral margins of temporal lobes parallel; two long setae at posterior margin of each lobe (Figure 33) . . . . . (the fluff louse [on chickens]) *Goniocotes gallinae*

9′ Lateral margins of temporal lobes distinctly angulate, not parallel; two or three long setae at posterior margin of each lobe, a short spine at angular process (Figure 34) . . . . . 10

10(9′) Two long setae at posterior margin of each temporal lobe . . . . . (the brown chicken louse) *Goniodes dissimilis*

10′ Three long setae at posterior margin of each temporal lobe (Figure 34) . . . . . (the large chicken louse) *Goniodes gigas*

11(7′) Anterior portion of head with a series of backward projecting chitinized processes (Figure 37) . . (the slender turkey louse) *Oxylipeurus polytrapezius*

11′ Anterior portion of head without such chitinized processes . . . . 12

12(11′) Dorsal posterior margin of pterothorax with four groups of long setae (Figure 38) . . . . (the chicken head louse) *Cuclotogaster heterographus*

12′ Dorsal posterior margin of pterothorax without groups of setae; posterior lateral margins of pterothorax each with a patch of long setae (Figure 39) . . . . . (the wing louse [on chickens]) *Lipeurus caponis*

### *Family Trichodectidae*

13(6′) Lateral margins of preantennal region straight, strongly convergent; apex of head with distinct narrow emargination (Figure 40) . . . . . (the cat louse) *Felicola subrostrata*

13′ Lateral margins of preantennal region rounded, arched; apex of head blunt, broadly emarginate or rounded (Figure 41) . . . . . 14

14(13′) Median transverse bands on abdominal tergites present; antennae normal (Figure 42) . . . . . 15

14′ Median transverse bands on abdominal tergites absent; antennae stout . . . . . (the dog biting louse) *Trichodectes canis*

15(14) Body sparsely covered with short setae . . . . . 16

15′ Body densely covered with long setae. . . (on angora goats) *Bovicola crassipes*

16(15) Head narrowing anteriorly, anterior margin sharply rounded or blunt (Figure 43) . . . . . (the cattle biting louse) *Bovicola bovis*

16′ Head having anterior margin broadly rounded or flattened (Figure 41) . . 17

17(16′) Anterior margin of head rounded, preantennal angle not markedly expanded; antennae long, basal segment of male antenna robust (Figure 44) . . . . . 18

17′ Anterior margin of head flattened or slightly concave; preantennal angles expanded, pointed, antennae same in both sexes (Figure 45) . . . . . 19

18(17) Width of temporal lobes about equal to that of preantennal region (Figure 44) . . . . . (the horse biting louse) *Bovicola equi*

18′ Width of temporal lobes less than that of preantennal region (Figure 46) . . . . . (the sheep biting louse) *Bovicola ovis*

*B. equi* and *B. ovis* are closely related species, their separation is quite difficult, generally the characters mentioned in the key will be useful.

19(17′) Anterior margin of head slightly flattened or concave in female (Figure 47), not so in the male; second abdominal tergite in male with a concave posterior border (Figure 48) . . . . . (the angora goat biting louse) *Bovicola limbata*

19′ Anterior margin of head slightly flattened or concave in male (Figure 47), not so in female; second abdominal tergite in male with a straight posterior border (Figure 49) . . . . . (the goat biting louse) *Bovicola caprae*

*B. limbata* and *B. caprae* have been considered distinct species by some authors; others consider them the same. *B. limbata* is more common on angora goats whereas *B. caprae* is more common on short-haired goats. The separation of the females of these species is practically impossible since only minor variation exists. The separation of the males is an easy matter because the genitalia of each species is characteristic for that species.

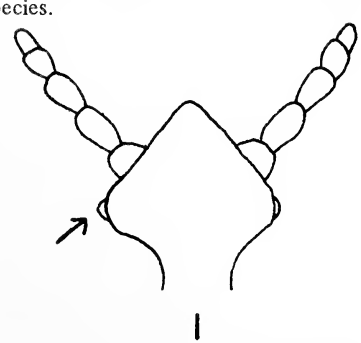
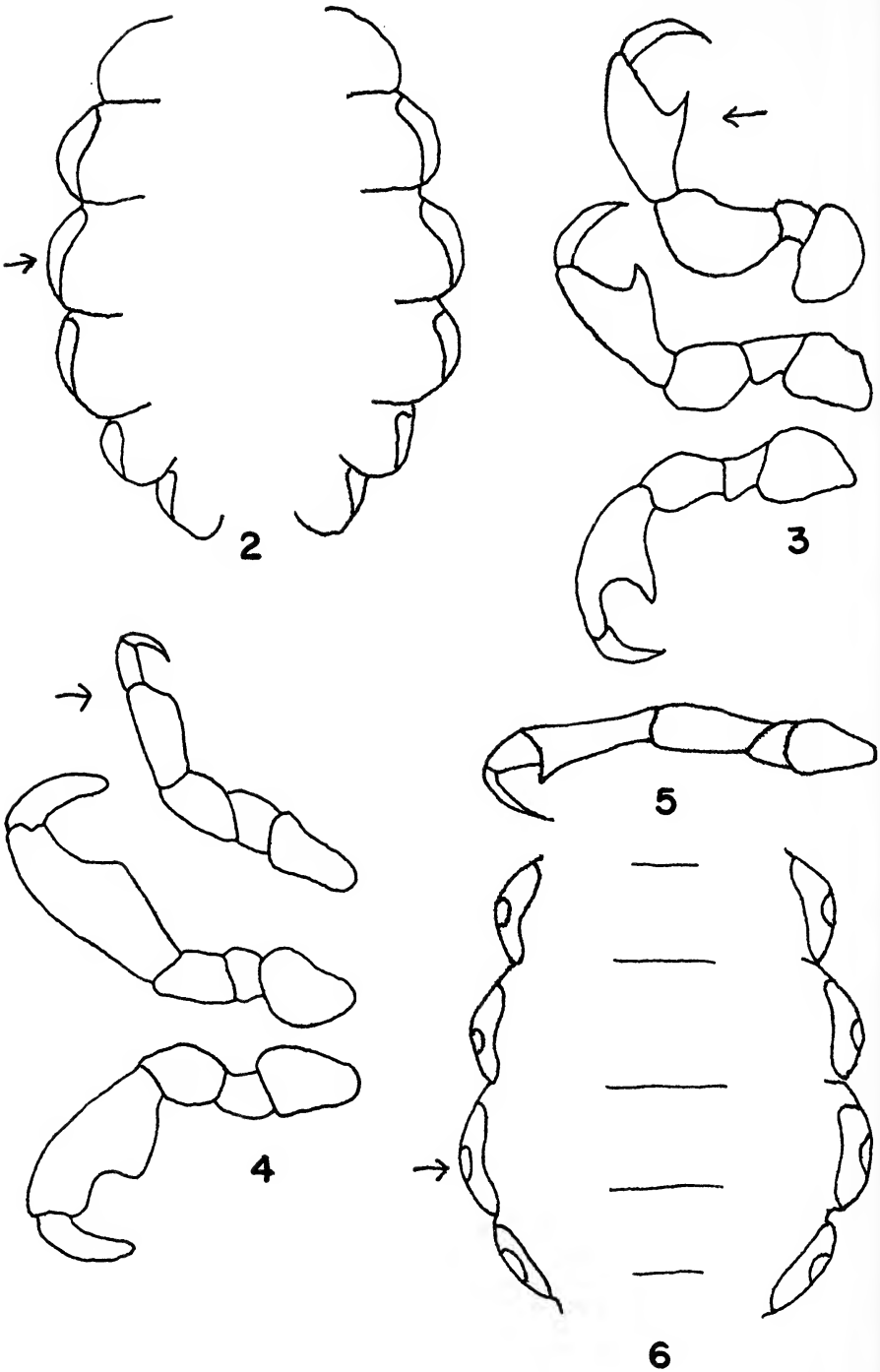


Figure 1. Illustration of diagnostic characters used in this key.




Figures 2-6. Illustrations of diagnostic characters used in this key.

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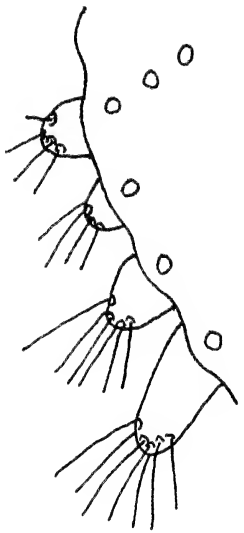

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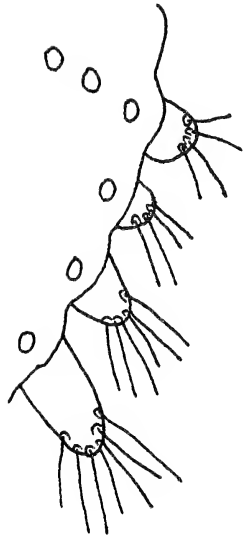

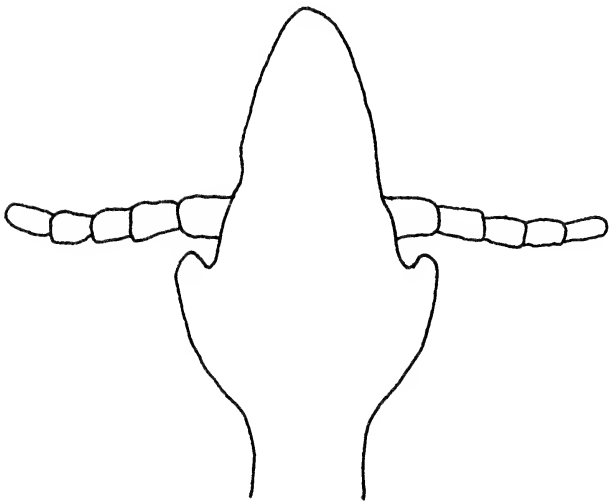

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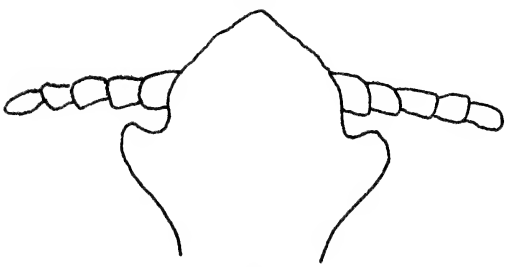

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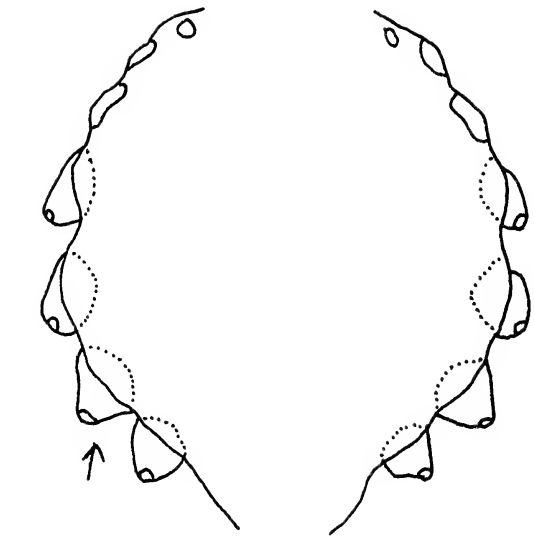
Figures 7-10. Illustrations of diagnostic characters used in this key.



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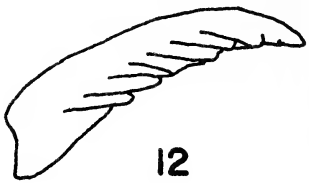

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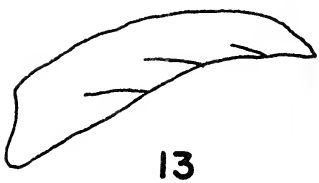

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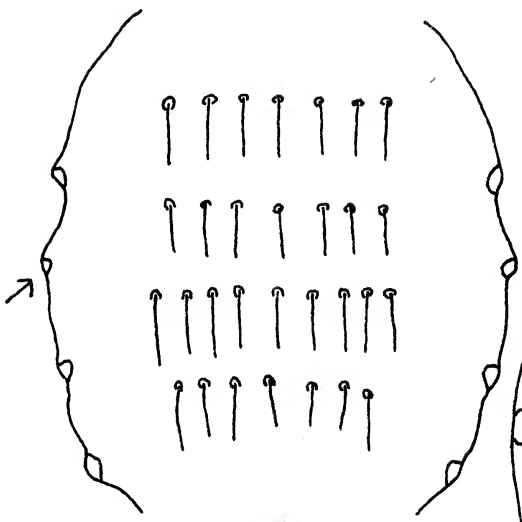

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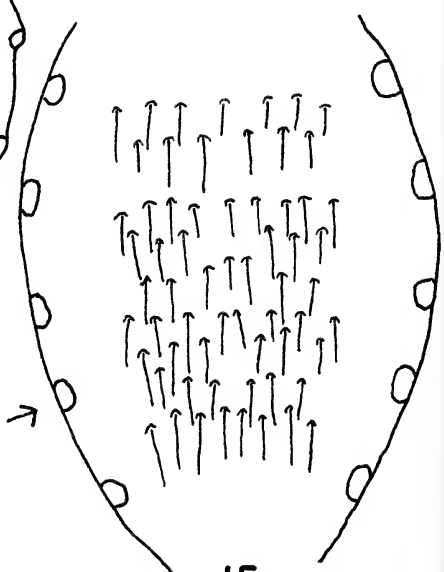

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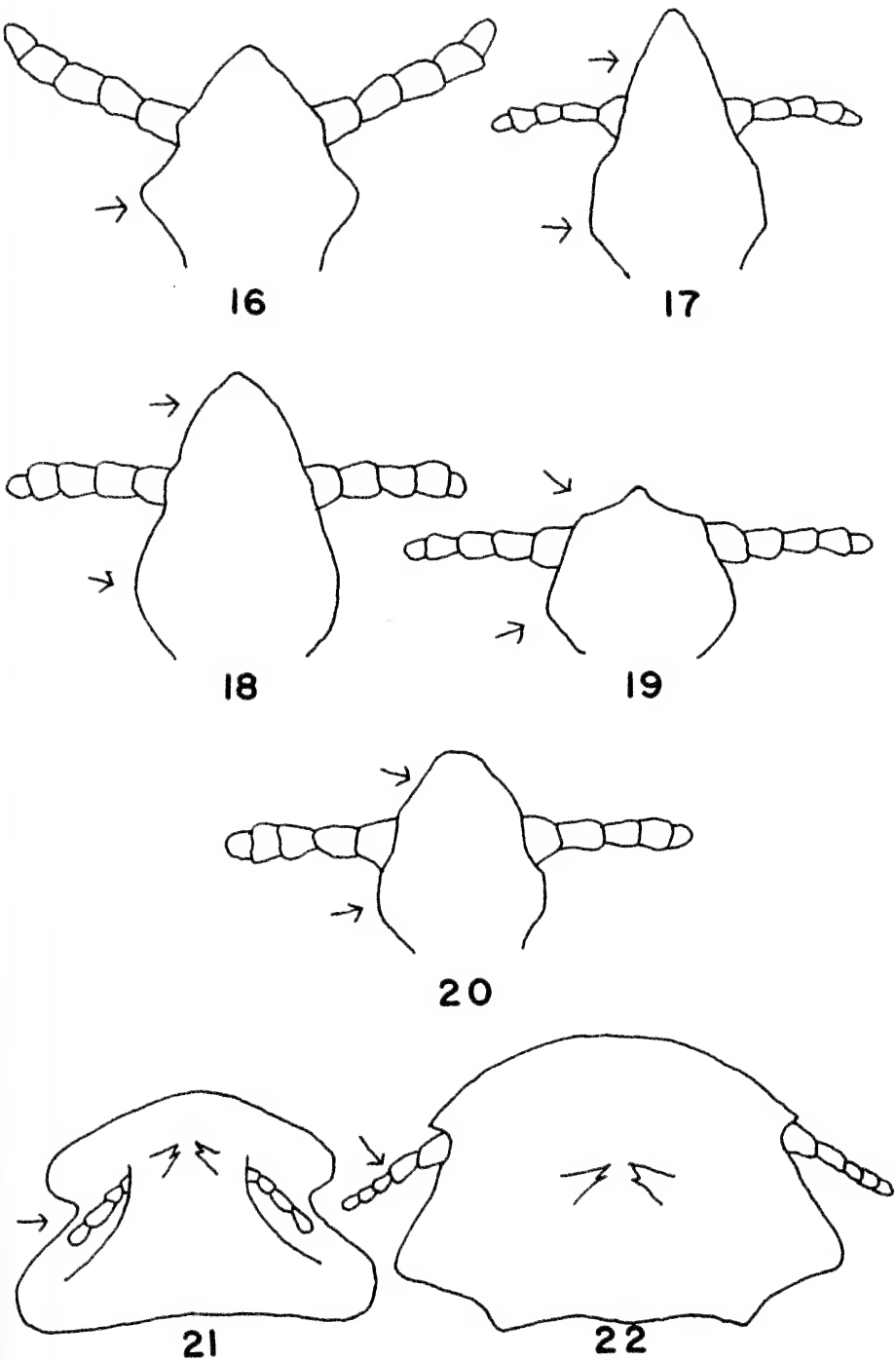
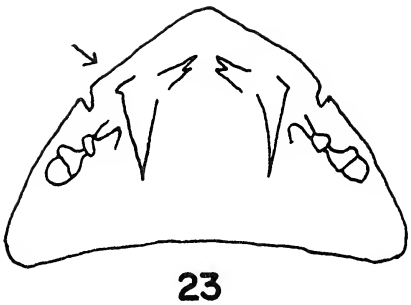
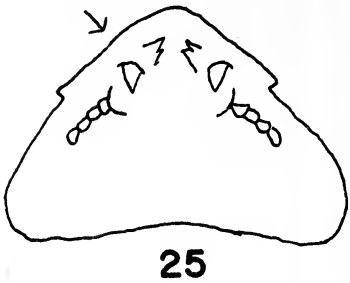
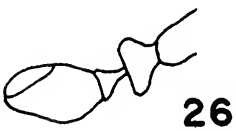
Figures 11-15. Illustrations of diagnostic characters used in this key.

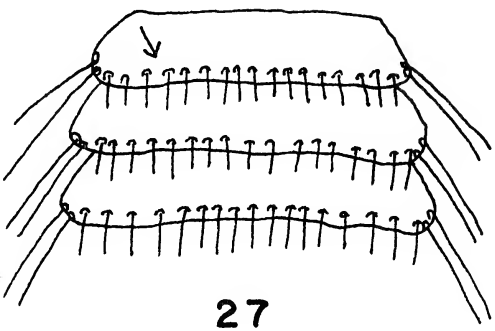


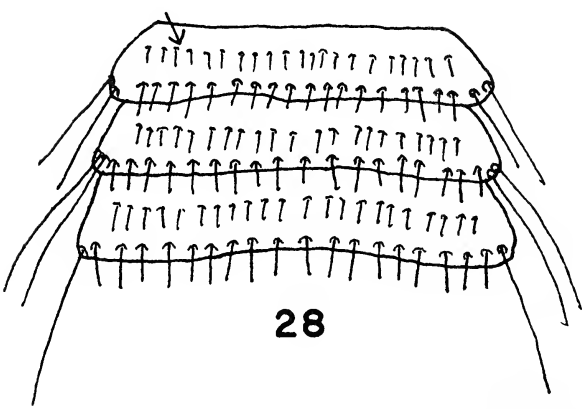

Figures 16-22. Illustrations of diagnostic characters used in this key.



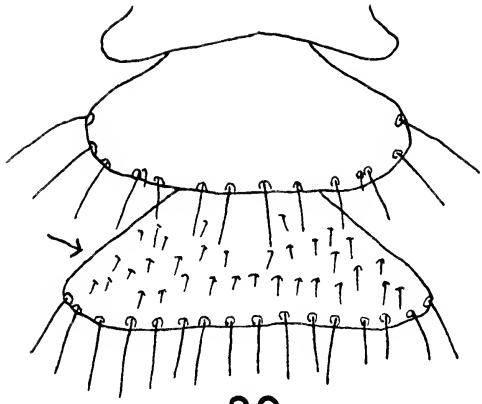






Figures 23-28. Illustrations of diagnostic characters used in this key.



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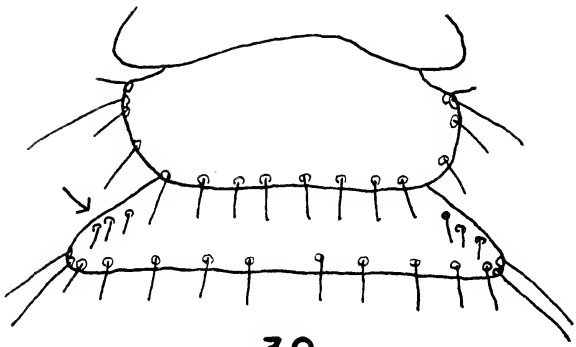

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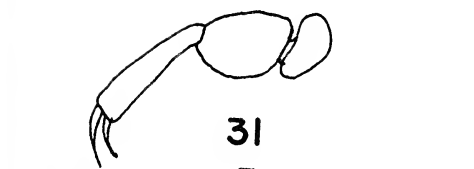

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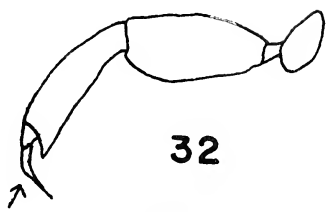

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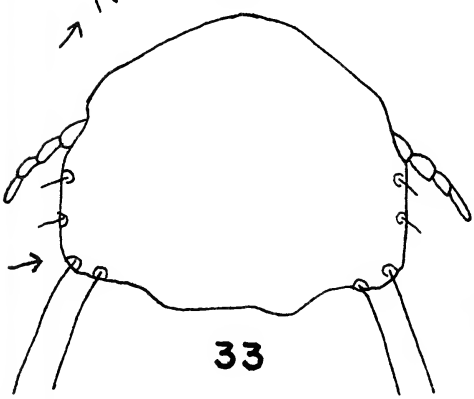

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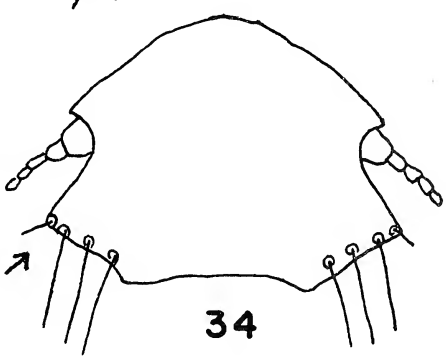

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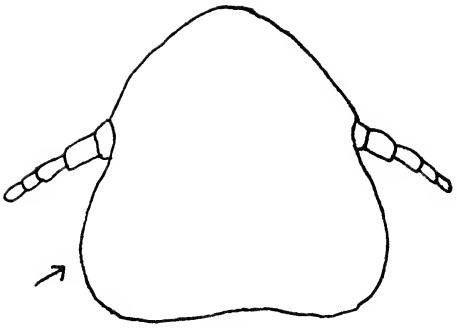
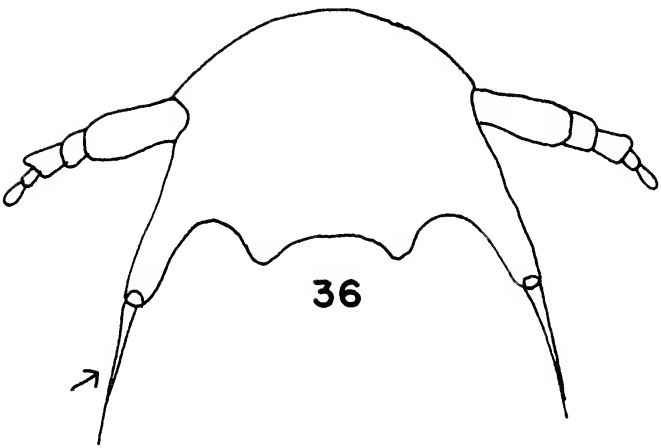
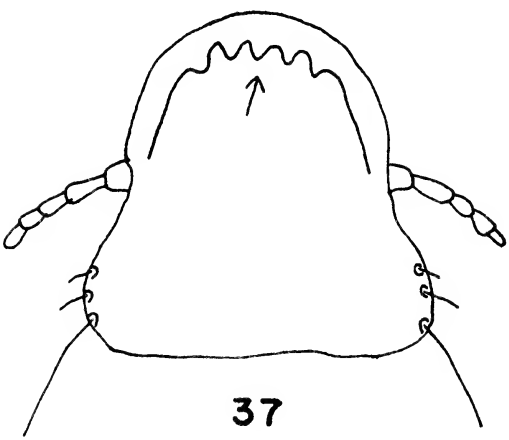
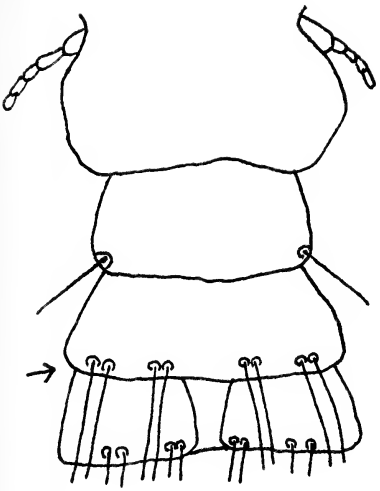
Figures 29-34. Illustrations of diagnostic characters used in this key.




Figures 35-37. Illustrations of diagnostic characters used in this key.



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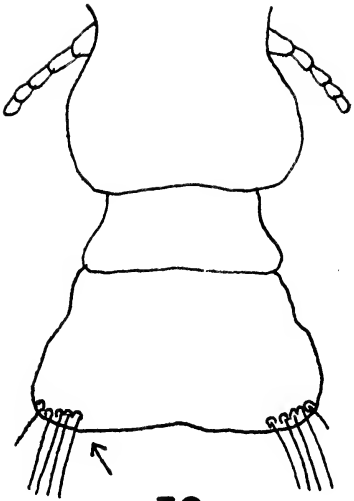

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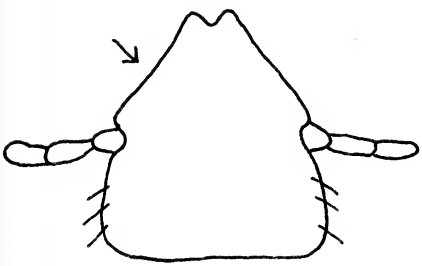

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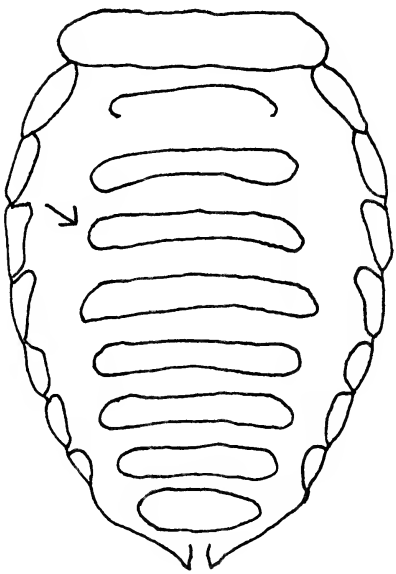

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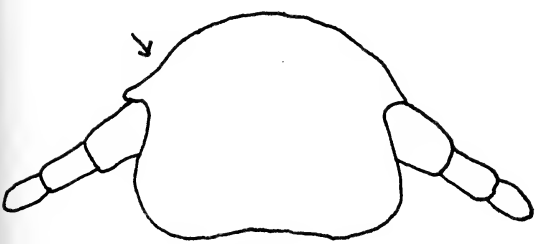

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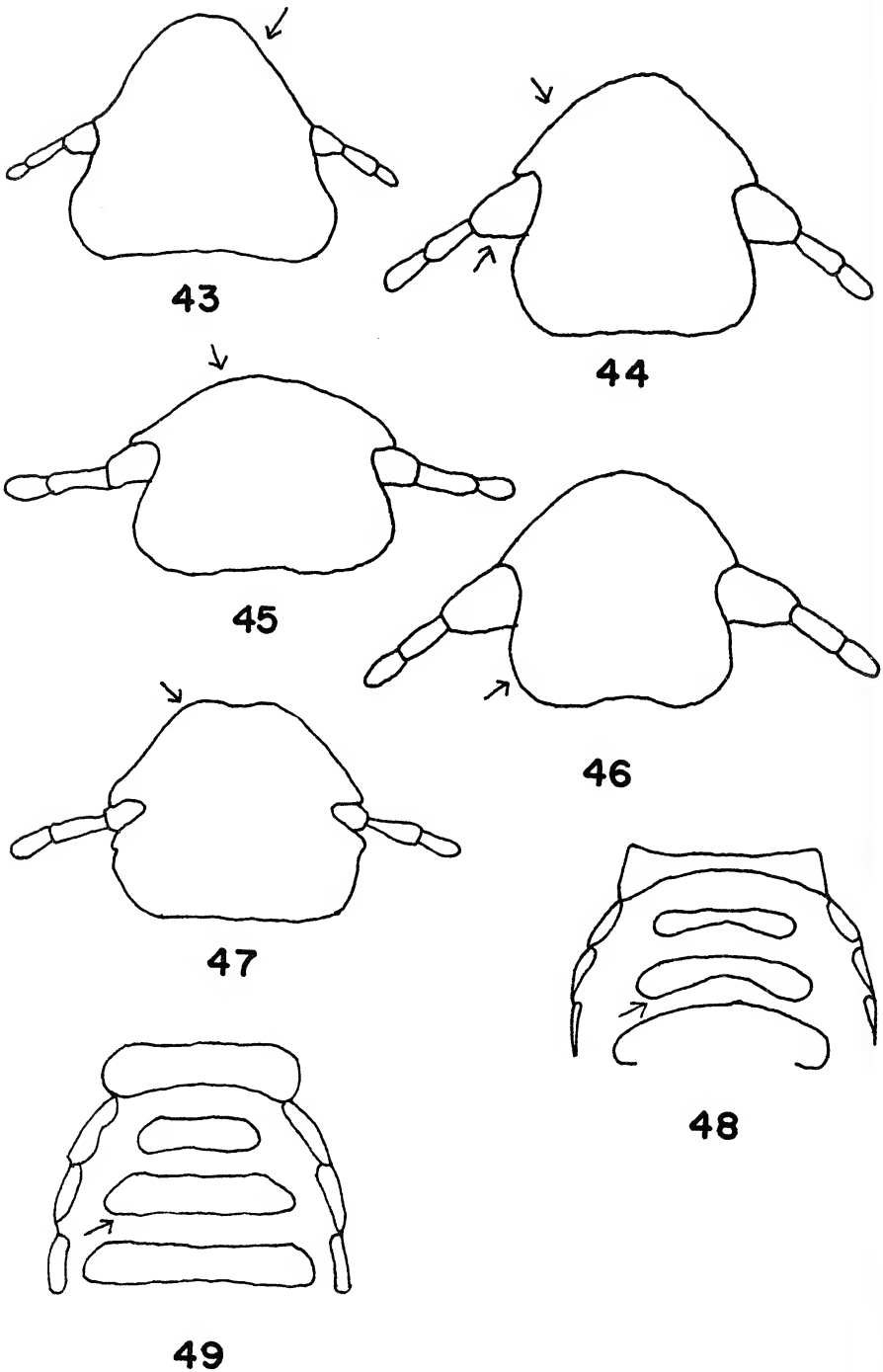
Figures 38-42. Illustrations of diagnostic characters used in this key.




Figures 43-49. Illustrations of diagnostic characters used in this key.

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# AN INDEX TO THE GENERA OF HOSTS IN YAMAGUTI'S *SYSTEMA HELMINTHUM*

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## INTRODUCTION

The first volume of Yamaguti's *Systema Helminthum* was published in 1958 and was immediately hailed by many parasitologists as a monumental first step toward systematically cataloging the helminths of the world. Unfortunately, none of the now completed five volumes contains an index to the hosts. Therefore, *Systema Helminthum* is most useful when used in conjunction with the host index of some other reference, such as the *Index Catalogue of Medical and Veterinary Zoology* or *Helminthological Abstracts.* However, both of these references are in serial form and their use under most laboratory conditions presents many logistical problems. Consequently, we have compiled this compact host index to compliment the use of *Systema Helminthum* in the laboratory.

This host index is divided into five sections corresponding to the five volumes of *Systema Helminthum.* The sections are arranged according to the usual taxonomic sequence, rather than in order of the publisehd volume number. Within each section, generic names of hosts are listed alphabetically, followed by page number in the appropriate volume.

Numerous synonyms and misspellings of host genera were detected in Yamaguti's text and were corrected in the index, but undoubtedly many more were transcribed undetected. When synonymies were suspected, a number of modern taxonomic references were consulted to determine the currently recommended name. We omitted the parasites of "dog," "cat," "man," etc., because page references to the well-known fauna of these hosts would be so extensive as to be virtually useless. The reader may encounter similar difficulties with such large and abundant genera as *Anas, Larus,* and *Rana.*

We wish to emphasize that Yamaguti did not attempt to include complete host lists in *Systema Helminthum.* Consequently, the reader is cautioned not to draw conclusions regarding new host records based on the exclusive use of this index.

## ACKNOWLEDGEMENTS

We wish to thank Interscience Publishers for permission to condense and publish this information. Southwest Texas State University is to be acknowledged for

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# *MONOGENEA and ASPIDOCOTYLEA (vol. IV)*

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(Cacatua-759) (Caccabis-755,759,764) (Cacicus-755,757,758,763) (Caesio-151,298,307) (Caiman-468, 482,487,489,536,537,541,543,544,545,546) (Cairina-572,628,694,695,780,784,797) (Calamus-33,37,38,47,110, 115,117,124,125,140,145,147,214,265,294,308,368) (Calidris-617,651,673,674,728,729,730,732,777,796) (Callichrous-44,86,122) (Callichthys-565) (Callinectes-730) (Callionymus-13,18,52,104,117,118,270,291, 293,371) (Callorhinus-861) (Callyodon-105,159,201,292,353) (Calopteryx-394,408) (Calospiza-755) (Calotes-431,472,474,475) (Calotomus-105,117) (Caluromys-834) (Cambaroides-932) (Cambarus-129,243, 379,396,483,553,932) (Camelus-835,937) (Campeloma-406,602,679,890,929) (Campostoma-118) (Campyloramphus-760) (Canachites-682) (Cancer-729) (Cancroma-687,702,869) (Canis-565,597,705,809, 835,857,859,863,867,869,871,874,878,885,897,899,934,943,945,946,947) (Cantharus-62,145) (Cantherines-36, 145,151,170,293,364,371) (Capella-570,607,626,627,644,647,652,727,729,732,742,765,773,774,776,787,788,893) (Capitella-727) (Capoetobrama-8,588) (Capra-835,841,957,959,972,973) (Capreolus-926) (Caprimulgus-615, 741,743,748,750,762) (Caranx-9,10,12,18,19,20,33,40,42,43,71,74,80,86,88,154,157,169,207,212,213,214,215, 277,278,279,293,297,307,308,338) (Carapus-162) (Carassiops-579) (Carassius-64,65,86,87,224,230,249, 366,579,588,591,599,638,687,707,714,716,859,873,877,878,880,882,883,898,942,943) (Carbo-583,586,648,651,704) (Carcinides-727) (Carcinus-729) (Cardium-19,65,142,739) (Carduelis-681) (Caretta-462,465,487,501, 506,517,518,530) (Cariama-565,752) (Caridina-160,732) (Carinogammarus-120,166) (Carphibis-563,626) (Carpiodes-66) (Carpodacus-681,682) (Casarca-571,572,614,615,638,795) (Casmerodius-565,566,622,653) (Caspialosa-40,263) (Cassiculus-627,629) (Cassidix-754,757,758) (Castor-904,906,968) (Cataphractus-358, 362,363) (Cathaica-835) (Catharista-637) (Cathartes-564,565,571,637,705) (Catoptrophorus-671,727) (Catostomus-66,67,87,88,118,131,569,579,589,703,860,870,890) (Caularchus-110,120) (Caulolatilus-148,188, 189,308) (Caulopsetta-167) (Causus-436) (Cebidichthys-300) (Cebus-820,831) (Celestus-479) (Celeus-755) (Centrarchus-88) (Centriscops-160,170) (Centrocercus-676) (Centrolophus-149,280,302) (Centropristis-109,142) (Centropus-628,678) (Centroscymnus-186,196) (Cepaea-829,908) (Cephalopholis-157) (Cephalophus-957) (Cepola-13,40,277,284,289,298) (Cepphus-621,708) (Cerastes-485) (Ceratacanthus-125,142) (Cerataspis-276) (Ceratophora-474) (Ceratophrys-404) (Cerchneis-632,757,760) (Cercibis-629) (Cercocebus-829,832) (Cercopithecus-829,936,977) (Cerdocyon-831) (Cerithidea-671,715,723,740,794,879) (Cerithium-205,341,342) (Cerivoula-834) (Certhia-681) (Cervicapra-974) (Cervus-797,830,842,964) (Ceryle-592,593,594,595,596,597,620,640,646,665,745) (Chaenobryttus-131,138,245,360,687) (Chaenogobius-65, 130,160,224,260,286,867,873,882) (Chaetodon-33,42,49,69,75,114,352) (Chaetoclipterus-114) (Chaetogaster-66) (Chaetopteryx-101) (Chaeturichthys-371) (Chalcalburnus-65,588,591) (Chalcides-479) (Chalcinus-152,248) (Chalinura-161) (Chamaeleo-392,401,470,471,477,479,838) (Champse-537,541,546) (Channa-707,873) (Chanos-12) (Chaoborus-615) (Charadrius-569,630,681,727,728,729,733,756,780,782) (Charax-101) (Chascanopsetta-266) (Chasmagnathus-887,932) (Chatoessus-30) (Chaulelasmus-778) (Chaunax-265,289,294) (Cheilichthys-134,188,319,368) (Cheilinus-167) (Cheilodactylus-289) (Chelidon-615,616,744,761,763) (Chelidonias-580,648,789,899) (Chelidonichthys-58,109,117,118,212,281) (Chelidoperca-170)

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(Chelonia-432,462,465,479,487,495,496,497,498,499,500,501,503,504,505,506,507,511,512,513,514,515,518,524,526,528,552,557,558) (Chelopus-457,458,459,521) (Chelydra-445,457,458,459,463,483,507,514,516,521,522,529,544,553) (Chelys-554) (Chen-791,792,795) (Chenolopex-628) (Chenopsis-572,780,791) (Cherox-806) (Chettusia-787) (Chilomycterus-113,349) (Chilonycteris-817) (Chiloscyllium-83,84,115) (Chiltonia-806) (Chimaera-196) (Chionis-791,795) (Chirocentrus-207,288) (Chiroleptes-411) (Chironectes-856) (Chironius-438,440,476) (Chironomus-391,615,806,821) (Chiroxiphia-760) (Chitra-424) (Chlamydoselachus-196) (Chlidonias-570,580,632,648,688,711) (Chlorichthys-109) (Chlorophthalmus-170,266) (Choerodon-117,118) (Chondrostoma-102,588,882) (Chorinemus-17) (Chrionema-13) (Chroicocephalus-787) (Chromis-601,941) (Chrysemys-243,446,457,458,459,460,483,507,521,522,557) (Chrysichthys-687) (Chrysolophus-779) (Chrysophrys-12,37,62,102,121,235,280) (Cichlasoma-138) (Ciconia-212,565,591,602,618,642,644,649,687,703,742,869,882) (Cinclus-738,743) (Cinixys-479) (Cinnyris-645) (Cinosternum-544) (Cionella-829) (Cipangopaludina-894) (Circaetus-563,587,646,759,867) (Circus-563,565,566,567,578,585,586,587,591,602,644,646,665,670,670,692,695,754) (Cirrhitus-220,227) (Cistudo-459) (Citellus-806) (Citharichthys-14,304) (Clangula-588,599,658,664,727,739,778,783,791,795,796) (Clarias-157,172,182,191,245,690,873,878,880) (Clemmys-457,458,459,459,460,520,555) (Cleopatra-601,867,878,941,971) (Clethrionomys-832) (Clinocottus-110,157,272) (Cloelia-457) (Clupanodon-149,266,277) (Clupea-9,11,34,166,254,263,264,266,271,276,280,296,297,298,300,301,305) (Clupisoma-172) (Cobitis-8,65,102,224,230,588,694) (Cobus-938,954,957,974,979) (Coccothraustes-676,683,788) (Cochlearius-565) (Coelopeltis-479) (Coelorhynchus-14,39,142,161,170,270,290) (Coenagrion-614) (Colobus-829) (Coloeus-614,665,676,755,789) (Cololabis-371) (Colossoma-356,361,363) (Coluber-430,434,438,444,449,450,452,453,457,467,485,488,540) (Columba-633,634,638,660,676,678,703,708,720,788,869,874) (Columbigallina-676) (Colymbus-569,570,571,573,578,579,581,632,635,638,644,647,648,649,694,701,702,708,708,717,720,768,786,798,868,869,873,874,883, 884, 899) (Complecta-703) (Condylura-908) (Conger-17,18,108,166,214,264,281,294,304,307,308) (Connochaetes-938) (Constrictor-444,475) (Contia-478) (Coracias-564) (Coracina-583) (Coragyps-564) (Corbicula-89,893) (Corcorax-681) (Cordulia-393) (Coregonus-8,53,86,130,131,132,591) (Coreperca-883) (Corethra-404) (Coridodax-368) (Corone-757,761,763) (Corvina-200,235,238) (Corvus-564,599,600,611,614,627,628,629,630,645,646,647,649,662,665,666,669,670,675,676,677,681,692,693,696,749,754,755,758,763,787,788, 789, 893) (Corydalis-406) (Coryphaena-213,252,253,255,276,318) (Coryphaenoides-142,284) (Cosmetira-149) (Cottocomephorus-130) (Cottunculus-102,103,271,290,370) (Cottus-17,27,86,87,88,89,102,109,114,117,118,119,129,130,131,132,159,212,214,264,270,271,286,297,305,547,601,709,711) (Coturnix-615,669,676,681,703,751,754,763,764) (Cotyle-615,754,755,762) (Craterocephalus-579) (Cratinus-271) (Crax-763) (Creagrutus-228) (Crecisicus-628,629) (Creisson-880) (Crenicichla-102,228) (Crenilabrus-17,37,53,86,117,307) (Crepidula-202,651) (Crex-681,682,763) (Cricetus-807,937,948) (Crimata-93) (Crinia-632) (Cristivomer-131) (Crocethia-730,733,734,799) (Crocidura-809,830,907) (Crocodilus-468,482,491,537,538,543,546) (Crocothemis-390) (Crotophaga-586,627,628,630,646,744,758,759) (Cryptacanthodes-214,271) (Cryptobranchus-384,413) (Cryptoglaux-563) (Cryptotomus-205) (Crypturellus-789) (Ctenolabrus-108) (Ctenopharyngodon-878) (Cuculus-614,616,665,754) (Culex-615) (Culicoides-615) (Cumingia-62,651) (Cuncuma-799) (Cuon-963) (Curimatus-228) (Cyanocitta-681,754,755,761,762) (Cyanocorax-758,760,789) (Cybium-20) (Cyclagras-425,438,446,473) (Cyclas-379,633,651) (Cyclemys-458) (Cyclops-263,264,287,395) (Cyclopterus-121,264,286,294) (Cygnus-569,571,609,611,629,658,661,664,665,694,791,793,794,795) (Cymatogaster-37,71,76) (Cymbium-9) (Cynchramus-616) (Cynopolius-773) (Cynoscion-21,118,134,145,166,200,213,282,310,368) (Cyprinus-64,65,65,87,101,131,175,249,293,366,586,588,707,714,716,855,868,870,873,877,878,882,883,885,942,943) (Cypselurus-333) (Cypselus-615,753) (Cystignathus-403,404) (Cyttus-118,170,281)

(Dacelo-564,580) (Dacnis-755) (Dactylopagrus-117,272,368) (Dactylopterus-280) (Dactylosargus-349) (Dafila-570,609,611,664,727,733,778,791,792,793,794,923) (Daicocus-158) (Dampieria-148,168) (Daphila-734) (Daphnia-806) (Dascyllus-292) (Dasyatis-84,85,90) (Dasycottus-214,271,290) (Dasylophus-681) (Dasymys-938) (Dasyprocta-937) (Dasypterus-823,824) (Dasypus-837) (Dasyurus-903,908) (Decapterus-166) (Decodon-57,117,140) (Deirochelys-457) (Delichon-744) (Delma-475) (Delphinapterus-850) (Delphinus-846,848,851,852,856,859,876,951) (Dendrocalaptes-677) (Dendrocoelum-64,392) (Dendrocopus-616,681,763,789) (Dendrocygna-628,637,638) (Dendrodryas-374) (Dendroica-681,684) (Dendromas-681) (Deniscus-429) (Dentex-235,304) (Dermatemys-464,515,550,555,557) (Dermophis-459) (Deroceras-754,757,762,909,910,912) (Desmana-905,945) (Desmognathus-376,381,383,399,413) (Diabrotica-754) (Diacope-107,264) (Diagramma-69,115,120) (Dibranchus-289) (Dicamptodon-383,386,876) (Dicerus-971) (Dichoceros-585) (Dicholophus-565) (Dicotyles-968) (Dicrostonyx-908) (Dicrurus-616,648,667,743) (Didelphis-734,805,830,838,856,886,891,901,908,909,933,934,937,944,947,948,949,950) (Diemictylus-86,399,400,405) (Diodon-113,316,349,371) (Diomedea-705,871) (Diplacanthopoma-30,289) (Diplectrum-305) (Diplodus-117) (Discoglossus-485,856) (Discognathus-869,883) (Dissoura-660,652) (Disteira-488) (Distichodus-239) (Ditrema-104,159,167,213) (Doderleinia-57) (Donax-19,53,71,222,739) (Doras-358,363) (Dorcelaphus-797) (Dorosoma-118,297,701) (Draco-476) (Dreissenia-86,87) (Drepanopsetta-27) (Dromas-650) (Drymarchon-450,450,474) (Drymobius-438,539) (Dryobates-665,677,681,682) (Dryophylax-438) (Dugong-920,923,979) (Dumetella-779) (Duymaeria-37,297) (Duymaria-37) (Dyromys-811,838) (Dytiscus-388,391,392)

(Echinogammarus-159) (Echinorhinus-196) (Egretta-565,566,582,588,591,636,644,654,687,706,715,716,873,882,883) (Elagatis-213) (Elanoides-563) (Elaphe-426,431,449,479,541,558,950) (Elaphis-450) (Elaps-446) (Eleginops-63,74,270) (Eleotris-950) (Elephas-841,844,965,967,971,976) (Elestris-269) (Eleutheronema-880) (Eliomys-907,908) (Elliptio-12) (Eloisa-411) (Elosia-381) (Emballonura-821) (Emberiza-615,681,748,754,755,767,788,789) (Embiotoca-51,71) (Emerita-727) (Emoia-476) (Emozamia-673) (Empidonomus-762) (Emyda-436,480) (Emydura-422,437) (Emys-243,424,459,460,464,483,516,521,522,523) (Enallagma-380,383)

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(Enchelyopus-208) (Endophrys-120) (Engraulis-30,149,264,267,292,297,298) (Engyophrys-142) (Enhydra-851, 861,870,886) (Enhydris-431,547,950) (Enneacanthus-88,595) (Enophrys-281) (Ensis-651) (Eophona-676,754) (Ephemera-101,131,667,821) (Ephippiorhynchus-629) (Epicordula-666) (Epigonus-142) (Epinephelus-16,17,18,37,89,107,108,109,120,127,142,149,157,159,174,213,214,278,281,282,294,305,329, 333, 342, 867) (Epitheca-379,380) (Epomops-837) (Eptesicus-805,806,807,816,817,822,823,824,826,915) (Eques-163,169) (Equula-69) (Equus-937,971,976) (Eremophila-756) (Eretmochelys-462,465,496,497,498,499,500,501,505,526,530) (Erichthonius-222) (Erignathus-855) (Erimyzon-66) (Erinaceus-832,897,908,908,936) (Eriocheir-932) (Erolia-563,634,651,673,727,729,732,734,799) (Erythrolamprus-449) (Erythropus-563) (Esox-8,9,12,17,44,86,88,101,129,131,160,183,195,243,483,570,581,591,592,890,942) (Eteone-727) (Etheostoma-87) (Etrumeus-52) (Eucalia-129,578,592) (Eucinostomus-72,138,165,265) (Eucynopotamus-270) (Eudryas-474) (Eudynamis-586) (Euhadra-400,678) (Eulota-678) (Eumeces-479,480,950) (Eunectes-425,426,446,457,473,615) (Euomphalia-829,908) (Euparypha-677) (Euphagus-757) (Euphractus-937) (Eupomacentrus-110) (Eupomotis-12,87,131,138,195,360,589,595,622,687,688) (Eurota-908) (Eurycea-382,399) (Eurynia-9) (Eurynorhynchus-751) (Eurypyga-741,752) (Eurystomus-694,766,860) (Eutaenia-447) (Eutamias-806) (Euthynnus-12,42,276,305,308,314,325,326,327,328,334,335,339) (Eutropiichthys-20,21,89,106,229,233) (Euxenura-619,642,646,742) (Exocoetus-57)

(Falco-563,564,573,585,586,587,591,615,628,666,670,679,684,692,695,751,758,759) (Farancia-396,441,445, 457,488) (Felichthys-308) (Felis-597,837,838,855,856,857,859,863,869,871,874,877,878,897,934,940,945,950) (Fennecus-597,945) (Fiber-793,807,892,895,923,924,925,946,948,969) (Fistularia-57,143,213,304) (Florida-566, 704,733) (Fluminicola-118,812,814) (Fluta-132) (Fontogammarus-160,658) (Formica-829) (Formicarius-760,760) (Fossaria-459,570,841,842,957,968) (Francolinus-763,781) (Fratercula-708,729,738) (Fregata-575, 601,603,711,868) (Fringilla-615,665,681,683,755,763,767,789) (Fulica-570,591,599,612,626,627,628,629,630, 632,634,662,663,665,667,681,682,746,748,770,773,774,781,782,788,789,792,795,799) (Fuligula-609,633,660,661, 662,727,778,780,783,786,902) (Fulix-692) (Fulmarus-769) (Fundulus-12,70,131,138,152,165,184,589,592, 715,723,799,871,879,881) (Furcimanus-27,142) (Furnarius-760)

(Gadus-9,19,59,120,121,142,165,205,212,214,263,263,264,271,286,290,292,297,304,708) (Gagata-79) (Galaxias-159,160,241,579) (Galba-841,842) (Galbula-744) (Galeichthys-28,241,308) (Galeorhinus-256) (Galictis-565) (Gallicrex-694) (Gallinago-569,574,594,606,629,727,773,774,782,797) (Gallinula-569,594, 627,628,629,630,636,661,677,681,682,683,746,752,773,774,778,780,781,792,794,799) (Gallus-564,570,600,616,627, 633,634,646,665,666,669,670,675,678 681,694,694,708,723,749,779,788,792,793,794,797,874) (Gambusia-184,371, 579,601,638,645,714,723,798,867,878,941,942) (Gammarus-101,117,120,159,391,392,412,733) (Garrulus-564,665,676,681,742,746,755,755,761,763,767,788,789) (Garzetta-635,690) (Gasterosteus-103,121,305) (Gastrodonta-908,909,912,913) (Gastroidea-754) (Gasterosteus-119) (Gavia-638,647,649,662,697,698,703,708,874) (Gavialis-469,491,541) (Gavilan-583) (Gecko-474) (Gelochelidon-673) (Gemma-740) (Genetta-950) (Genypterus-304,306) (Geoclemmys-457,458) (Geoemyda-457,458,459,479,516) (Geothelphusa-931,932) (Geothlypis-684,743) (Gephyrocharax-182,228) (Gerbillus-675,908) (Germo-335) (Geronticus-628,629,647,648) (Gerres-138,190,207,722,868,877,878,880) (Gerris-722,868,877,878) (Gibbonsia-110,120,170) (Gillichthys-723) (Ginglymostoma-84) (Giraffa-841) (Girella-148,149,157,205,292) (Glanidium-119) (Glareola-741,743,748) (Glaucidium-563,758) (Glaucionetta-570,649,693,708,728,729,735,778,792,795,874) (Glirulus-824) (Glis-908) (Globiocephalus-848,849,853) (Glossogobius-18,157,195,225,226,269,277,645,707,720,873,877,878,880,884) (Glottis-773,774,776,779) (Glyptanisus-960) (Glyptocephalus-212) (Glyptosternum-172) (Gnathonemus-689) (Gnathopogon-65,118,224,230,260,707,716,873,883) (Gobio-87,117,588,860,869) (Gobiomorphus-125,159,241) (Gobius-10,17,53,65,80,102,108,109,160,166,167,286,293,304,307,708,709,874) (Gomphus-390,666) (Goniistius-105,107,115,151,157,293) (Goniobasis-118,121,198,376,506,507,749,870,874,888) (Gorsakius-644,716,883) (Goura-651) (Grallina-564) (Graptemys-483,507,516,520,521,533) (Grayia-491,558) (Grison-838) (Grus-628,754,770,775,779,783,893) (Guara-704) (Guira-630,744,752,788) (Gulo-855,863) (Guttera-774) (Gymnacanthus-27,106,121,142,264) (Gymnarchus-78,87,235) (Gymnodactylus-475) (Gymnosarda-253) (Gymnothorax-11,17,304,307,308,309) (Gyraulus-375,406,409,582,614,626,891,893,894,897,925)

(Hadropterus-87) (Haematopus-627,651,658,728,732,733) (Haemopis-569,571) (Haemulon-33,47,57,62,69, 70,71,107,108,109,162,163,214,273,294,332,601) (Hagedashia-612) (Halcyon-573,595,596,661,754,765,773) (Haliaetus-563,564,571,585,586,587,591,695,705,710,882) (Haliastur-564,587,603) (Halichoeres-104,107,110, 160,205,297) (Halichoerus-855,859,863) (Halicore-919,920,922,978) (Halieutichthys-142) (Haminoea-614) (Hapalogenys-167) (Haplochromis-178) (Hardella-420,506,525) (Harelda-658) (Harengula-149,222,266, 277,298,701,868) (Harpagus-758) (Harpiprion-646,741,752) (Harpodon-280,351) (Hedimela-742) (Hedymela-762) (Hedymeles-757,761) (Helice-735,887,932) (Helicella-676,779,829,908,829) (Helicigona-908) (Helicodiscus-913) (Helicogena-829) (Helicolenus-109,165,169,170,277,281,283) (Helicoperca-87) (Helicopsis-676) (Helioperca-138,198,589) (Helisoma-66,183,184,245,263,371,379,395,405,406,458,459,463, 521,522,553,571,572,592,595,622,638,688,703,784,797,799,892,909,946,979) (Helix-676,681,908,908,909) (Helizella-914) (Helodrias-633) (Helodromas-616,632,776,794) (Helostoma-358) (Hemibarbus-65) (Hemichromis-942) (Hemiclepsis-89) (Hemiculter-880) (Hemidactylus-472,474,475) (Hemigrapsus-727,887) (Hemipipa-375) (Hemiramphus-56,311,722,868,880) (Hemitripterus-121,157,212,214,271,282) (Heosemys-556) (Hepsetia-342,722,868,880) (Herodias-565,566,582,588,628,635,648) (Herpailurus-838) (Herpestes-943) (Herpetodryas-448,551) (Herpetotheres-564) (Herpobdella-64,569,571,572) (Hesperiphona-754) (Heterandria-184) (Heterodon-449,450,450,452,617) (Heterodontus-90) (Heteropneustes-44,45,164,172,191,192) (Heteropygia-696) (Heterotis-490) (Hexagenia-131,152) (Hexagrammos-60,115,157,272) (Hexanchus-196,197) (Hieracidea-563,646,692) (Hieraetus-581,584) (Hierococcyx-567)

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(Himantopus-649,671,702,727,729,741,752,773,777,778,781,782,784,787,789,794,797,798,869) (Hiodon-14,131) (Hippeutis-897) (Hippocampus-212,215) (Hippoglossoides-53,55,106,165,212,271) (Hippoglossus-30,55,212,237,263,270,271,272,290) (Hippopotamus-956,958,960,961,962,963,974) (Hippotragus-918, 938,958,974) (Hirundo-615,616,665,742,744,766,768) (Histrionicus-737,792) (Holacanthus-34,360) (Holconotus-162) (Holocentrus-126,134,164,170) (Homalonyx-682) (Hoplerythrinus-689) (Hoplias-244,689) (Hoplichthys-13) (Hoplognathus-28,29,57,115,127) (Hucho-195) (Huro-87,131,223,366,547,589,601) (Huso-211) (Hyalella-121) (Hyborhynchus-118,582,589,856) (Hydraspis-549,554) (Hydrobia-65,268,694) (Hydrochelidon-578,607,615,616,632,649,681,768,787,788) (Hydrochoerus-924,928,968) (Hydrocoloeus-606,607,613) (Hydromys-806,886,949) (Hydroprogne-573,578) (Hydropsalis-627) (Hydroscopus-457) (Hyelaphus-963) (Hyla-14,64,307,374,375,376,379,380,381,388,391,392,399,400,401,403,408,411,429,445,449,454,478,479, 548, 565, 571,629,632) (Hylophylus-759) (Hynnodus-149,265) (Hynobius-892,893) (Hyodon-118) (Hypentelium-66,118) (Hyperoodon-851) (Hypleurochilus-114) (Hypoclydonia-12) (Hypodytes-109) (Hypolais-789) (Hypomesus-260) (Hypomorphus-586) (Hyporhamphus-34,205,297,371) (Hypotaenidia-645,682,699,742) (Hypotriorchis-563,567,615,616,754,755) (Hypsoblennius-114)

(Ibis-600,614,646,648,667,742,765) (Icelus-118) (Ichthyoborus-647) (Ictalurus-112,152,184,185,226, 243,245,483,547,601,870) (Icteria-745) (Icterus-564,682,758,789) (Ictiobus-66,199,337) (Ictis-946) (Iduna-615,789) (Idus-175,581,855,856,882,898,942) (Iguana-465,492) (Iheringichthys-132,366) (Ilyanassa-51,727) (Ilybius-404) (Indoplanorbis-895,937,938,973,976) (Inimicus-18,157,270,281,304) (Ionornis-795) (Iridio-109) (Ischnura-383) (Istiophorus-267) (Ixobrychus-566,573) (Ixoreus-747)

(Jabiru-591,742) (Jacana-639,752,773) (Jenynsia-591) (Johnius-212,278) (Jordanella-184) (Julis-37) (Junco-760)

(Kachuga-424,425,426,427,458,459,480,501,506,508,525) (Kamimuria-816,824) (Katayama-932,937) (Kathetostoma-9,10) (Katsuwonus-330) (Ketupa-579,714) (Kinixys-950) (Kinosternon-457,458,459,516,529) (Kirkaldia-390) (Kittacincla-586) (Kyphosus-135,136,205,250,300,369)

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(Umbrina-43,76,82,108) (Upenichthys-107) (Upupa-135) (Uria-71,86) (Urocissa-125) (Urocyon-138,142)

(Vampyrus-131) (Vanellus-71,73,90,124,151,152) (Varanus-128) (Varicorhinus-94,95) (Verasper-41) (Vimba-42,44) (Vipera-80,122,125,126,129) (Vulpes-130,141,143) (Vultur-118)

(Wilsonia-14)

(Xenarthra-138) (Xenocypris-47) (Xenodon-135,136) (Xenopeltis-136) (Xesurus-53)

(Zacco-19,21,47,66,67) (Zalophus-80,82) (Zamenis-122) (Zanclorhynchus-42) (Zebrias-50) (Zebrius-96) (Zeus-158) (Zoarces-39,42,44,94,96) (Zosterops-119)

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# EFFECTS OF PROMETRYNE ON RNA AND PROTEIN METABOLISM IN BEAN LEAF DISCS

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ABSTRACT

The effects of 2,4-bis (isopropylamino)-6-methylmercapto-*s*-triazine (prometryne) on RNA and protein metabolism in bean leaf discs were studied over a period of 7 days. Prometryne was found to have no effect on the RNA content of the discs or RNA synthesis as measured by $^{14}$C-uracil incorporation during the 7 day treatment period. In contrast, the protein content of the discs declined significantly by the 5th day of treatment, and a significant reduction in protein synthesis as measured by $^{14}$C-alanine incorporation was observed at the same time. The data did not reveal a specific site(s) of action of prometryne on protein synthesis, but did suggest an increased rate of senescence of the discs, probably due to blockage of the photosynthetic mechanism.

## INTRODUCTION

The primary effect of 2, 4-bis (isopropylamino)-6-methyl-mercapto-*s*-triazine (prometryne) and other substituted *s*-triazine herbicides is generally believed to be on photosynthetic reactions (Gysin and Knusli, 1960). Reduction of $CO_2$ fixation in plants treated with *s*-triazines has been shown, (Ashton, *et al.*, 1960; Sikka and Davis, 1969; Smith and Bucholtz, 1962; Zweig and Ashton, 1962) and the effect of these herbicides on the Hill reaction of isolated chloroplasts has been well documented (Gysin and Knusli, 1960; Good, 1961; Hilton, *et al.*, 1963; Moreland, *et al.*, 1959; Moreland and Hill, 1962). Although *s*-triazines unquestionably affect photosynthetic reactions it is possible that such effects are secondary in the intact plant, and a close relationship between protein metabolism and photosynthetic activities has been suggested (Rhodes and Yemm, 1963). Prometryne has been found partially to replace uracil in *E. coli* RNA (Moreland, 1967), Singh and West (1967) have reported that 2-chloro-4, 6-bis (ethylamino)-*s*-triazine (simazine) inhibits RNA and protein synthesis in oat chloroplasts and Beruter and Temperli (1970) found that prometryne inhibits RNA synthesis before $CO_2$ fixation

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is affected in spinach. Ashton, *et al.*, (1963) reported that 2-chloro-ethylamino-6-isopropylamino-*s*-triazine (atrazine) severely altered the structure and morphology of kidney bean chloroplasts, even to the extent of their complete destruction. McDaniel and Frans (1969) have found that prometryne inhibits electron transfer and oxidative phosphorylation in soybean mitochondria in a manner similar to oligomycin. However, Mann *et al.*, (1965) found no effect of atrazine on protein synthesis in barley and *Sesbania* seedlings, Moreland *et al.*, (1969) reported that atrazine did not affect RNA and protein synthesis in excised corn and soybean tissues, and Truelove *et al.*, (1973) obtained questionable results concerning an effect of prometryne on protein synthesis in cucumber leaf discs. Thus the available information is somewhat contradictory concerning the effects of *s*-triazines on RNA and protein metabolism in plant tissues.

This paper reports the effects of prometryne on RNA and protein metabolism in bean leaf discs.

## MATERIALS AND METHODS

### *Plant Materials*

Kidney beans (*Phaseolus vulgaris* L.) were planted in flats of sterilized vermiculite, watered as needed with either tap water or Hoagland's solution, and grown in the greenhouse for 14 days. The primary leaves were excised, washed in deionized water, and discs 1 cm in diameter were punched from the inverveinal areas of the leaves. Groups of 10 discs (average total weight of 0.125g) were floated on 15 ml of the appropriate aqueous solution contained in petri dishes. The dishes were placed in a vacuum desiccator, subjected to a negative pressure of 10 mm Hg for 20 min to infiltrate the tissue, and transferred to the laboratory bench under a 24 hr photoperiod of approximately 1,000 lux from cool-white fluorescent lamps at a temperature of 26 C. Five sets of 10 discs each were frozen for zero time analysis, and 5 replications of each treatment were removed at 1, 2, 3, 5 and 7 days and frozen until analyzed.

### *Treatments*

The basal solution consisted only of water that contained 20,000 units/liter of penicillin. Solutions used to determine the effect of prometryne on RNA and protein content of the leaf discs consisted of the basal solution made to 16.6 $\mu$M with prometryne. Synthesis of RNA was studied by following the incorporation of $^{14}$C-uracil in discs floated on solutions composed of basal solution, 16.6 $\mu$M prometryne and 1$\mu$c $^{14}$C-uracil (Amersham/Searle, sa 50 mc/m mole). Solutions used to follow protein synthesis contained 1 $\mu$c $^{14}$C-alanine (Amersham/Searle, sa 105 mc/m mole). Control solutions contained no prometryne, but were otherwise identical. Kinetin, which is frequently incorporated in solutions used in studies of this type, was not included because Goldthwaite and Laetsch (1967) have reported it to be relatively ineffective in retarding senescence in bean leaf discs, whereas white light was very effective.

*Analytical Procedures*

Protein and RNA were extracted by a modification of the techniques of Barker and Hollingshead (1964) and Osborne (1962). The replicates of 10 discs were homogenized in a minimum volume of 80% ethanol and centrifuged at 2000 xg for 5 min. The residue was washed once with 1 ml of 95% ethanol saturated with sodium acetate, and then suspended in 5 ml of ice cold 0.2N perchloric acid for 10 min. The precipitate was recovered by centrifugation as before, washed twice with 1 ml cold 0.2N perchloric acid, once with 1.5 ml 95% ethanol saturated with sodium acetate, and twice with 3 ml of a mixture of 95% ethanol saturated with sodium acetate and ether (3:1 v/v). The residue was suspended in 2 ml 0.3N KOH, incubated for 1 hr at room temperature, centrifuged, and the supernatant solution was saved. The pellet was extracted again and the 2 supernatant fractions were combined. The residue was reserved for determination of insoluble protein. The combined supernatants were divided into 2 aliquots, one for determination of RNA and the other for determination of soluble protein.

The RNA aliquot (2 ml) was prepared by adding 1.3 ml of 1N perchloric acid and allowing the solution to stand in ice for 10 min. The pellet recovered by centrifugation as before was washed once with 2.7 ml of 0.2N perchloric acid, and the washing was added to the first supernatant. The total RNA was determined by absorption measurement at 260 nm in a Beckman DU spectrophotometer. The amount of RNA present was calculated on the basis of a standard curve prepared from yeast RNA (Sigma Chemical Company).

Soluble protein was determined by the method of Lowry *et al.*, (1951). The residue was treated with 1N NaOH at 100 C for 4.5 min to render the insoluble protein soluble, and determinations were made as before. The amounts of protein present in these samples were calculated on the basis of a standard curve made by using bovine serum album.

The activities of the soluble protein, insoluble protein, and RNA fractions were determined by liquid scintiallation counting. The counts were corrected for quenching.

## RESULTS

*Extraction of RNA*

The method of RNA extraction employed in this study effectively removed all RNA from the tissue as determined by liquid scintillation counting and absorbance at 260 nm. Extraction for a longer time (Osborne, 1962) or with 1N NaOH according to Barker and Hollinshead (1964) gave greater absorption at 260 nm, but the higher readings were due to gross contamination of the samples by protein soubilized by the treatments. Corrections for protein absorbance according to Barker and Hollinshead (1964) gave similar readings by the 3 methods.

*Effect of Prometryne on RNA Content and Synthesis*

The data in Table 1 indicate that prometryne had no effect on either RNA content of the leaf discs or RNA synthesis as measured by the incorporation of $^{14}$C-uracil. All values are within the standard deviation of the means, thus no significant differences can be attached to the slight variations.

TABLE 1

Effect of Prometryne on RNA Content[a] and $^{14}$C-uracil Incorporation[b] in Bean Leaf Discs.

| Day of Treatment | RNA Content | | $^{14}$C-uracil Incorporation | |
|---|---|---|---|---|
| | $H_2O$ | Prometryne | $H_2O$ | Prometryne |
| 0 | 133±17 | 133±17 | – | – |
| 1 | 180±28 | 166±26 | 40±8 | 41±9 |
| 2 | 189±25 | 175±34 | 42±7 | 47±6 |
| 3 | 188±18 | 216±39 | 41±7 | 40±3 |
| 5 | 207±39 | 194±15 | 39±5 | 34±4 |
| 7 | 149±25 | 147±11 | 30±6 | 32±2 |

[a]Data are expressed as μg RNA/10 discs and are the average of 5 replications.
[b]Data are expressed as cpm/μ RNA, and are the average of 5 replications.

*Effect of Prometryne on Protein Content and Synthesis*

Protein content or synthesis in bean leaf discs does not appear to be affected by prometryne until after 3 days of treatment. If the means and standard deviations as shown in Table 2 are considered, the prometryne treated discs

TABLE 2

Effect of Prometryne on Total Protein Content[a] and $^{14}$C-alanine Incorporation[b] in Bean Leaf Discs.

| Day of Treatment | Protein content | | $^{14}$C-alanine Incorporation | |
|---|---|---|---|---|
| | $H_2O$ | Prometryne | $H_2O$ | Prometryne |
| 0 | 693±45 | 693±45 | – | – |
| 1 | 756±72 | 862±43 | 191±45 | 126±32 |
| 2 | 762±75 | 692±81 | 195±37 | 133±20 |
| 3 | 817±74 | 715±72 | 228±50 | 159±18 |
| 5 | 837±70 | 602±38 | 170±29 | 121± 2 |
| 7 | 809±50 | 596±34 | 173±19 | 106± 9 |

[a]Data are expressed as μg protein/10 discs, and are the average of 5 replications.
[b]Data are expressed as cpm/μg protein, and are the average of 5 replications.

contained a minimum of 17% less protein at both the 5 and 7 day sample periods. Although the prometryne treated discs tend consistently to incorporate less $^{14}$C-alanine into protein, again the differences are not significant until

after 3 days of treatment. The data in Table 2 indicate a minimum inhibition of protein synthesis in the treated discs of 13% at 5 days, and 25% at 7 days.

## DISCUSSION

The data indicate that prometryne had no effect on the total RNA content or synthesis of RNA as measured by incorporation of $^{14}C$-uracil in bean leaf discs under the experimental conditions employed. Such results are not in agreement with those of Berüter and Temperli (1970). These investigators found that total RNA synthesis, as measured by $^{32}P$ incorporation in intact spinach plants grown in solution culture, was reduced within a few hours after treatment with prometryne. Singh and West (1967) have shown that a reduction of total RNA in simazine treated oat plants cannot be detected by 6 days after treatment, but that the synthesis of chloroplastic RNA was reduced by that time. It is possible, therefore, that prometryne may be more specific in its action in beans than in spinach, and that minute changes in RNA content or synthesis in organelles such as chloroplasts could not be detected by the techniques employed. However, the data of Singh and West (1967) do show a slight reduction in total RNA content after 7 days of treatment, and a continued decline thereafter. Thus the results presented in the present paper are interpreted to indicate no effect of prometryne on RNA synthesis in bean leaf discs.

The data concerning protein metabolism are more difficult to interpret. Several possible explanations exist, but the data do not definitively indicate whether a primary mechanism is involved, or whether the decline in protein content and synthesis is the result of a combination of mechanisms. The slow onset of significant symptoms (5th day of treatment) suggests that the prometryne does not directly affect protein synthesis, but rather the effect is upon some cofactor(s) or intermediate(s) essential for protein synthesis. The data presented in this paper suggest that RNA is not a limiting factor as a decrease in protein synthesis would be expected to be preceded, or at least accompanied, by a decrease in RNA content and synthesis. Protein synthesis requires high-energy phosphate compounds, and ATP is essential for some steps. Thus blockage of oxidative phosphorylation as shown by McDaniel and Frans (1969) is one possible site of action for prometryne, but does not appear to be the main one since ATP is an essential nucleotide for RNA synthesis. A reduction in ATP concentration that would inhibit protein synthesis would probably inhibit RNA synthesis also.

Senescence in plant tissues is characterized in part by a decline in protein synthesis and content (Goldthwaite and Laetsch, 1967; Osborne, 1962). Goldthwaite and Laetsch (1967) found that the ability of white light to retard senescence in bean leaf discs was blocked by concentrations of 3-(3,4-dichlorophenyl)-1, 1-dimethylurea that inhibited photosynthesis. Because prometryne

is a known inhibitor of $CO_2$ fixation (Sikka and Davis, 1969), the most probable explanation for its effect on protein metabolism in bean leaf discs is an accelerated rate of senescence due to blockage of the photosynthetic mechanism. Senescence might also be expected to result in decreased RNA synthesis (Goldthwaite and Laetsch, 1967); thus the data presented here further suggest that pathways for protein metabolism are affected more rapidly than pathways for RNA metabolism in bean leaf discs.

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# RESULTS OF A MAGNETOMETER SURVEY AT HUECO TANKS, A PREHISTORIC MOGOLLON VILLAGE IN WESTERN TEXAS

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ABSTRACT

Results of a magnetometer survey at Hueco Tanks State Park (41 EP 2) suggest a relationship between magnetic anomalies and pit house structures and other areas of cultural activity at this site. Hueco Tanks is a late prehistoric site of the Jornada Branch of the Mogollon near El Paso, Texas. Two techniques of magnetometer surveying were employed. One consisted of a tightly controlled "insite" mode. The data from this technique can be reduced to a map, which shows the variations in magnetic intensity by contour lines. The second method employed a "search mode" technique of a more wide-ranging nature. Anomalies discovered by the proton magnetometer were tested by excavation.

## INTRODUCTION

This survey used the magnetometer to locate cultural features for excavation and to help define the extent of the village area. The survey and subsequent excavation results are discussed following background information on the instrument and the site.

The magnetometer can be an important tool for archeology, both on land and underwater. It is a remote sensing device which enables the archeologist to make a preliminary assessment of the sub-surface cultural features of a site without excavation. Data from a careful magnetometer survey can be invaluable in planning research strategy and in choosing excavation units. Underwater archeologists of the Texas Antiquities Committee have successfully used the magnetometer to produce magnetic contour maps of mid-16th century Spanish shipwrecks off the Texas coast. These maps are used in association with shore-based control system to direct the excavation in the murky water (Clausen and Arnold, in preparation). Interest in the application of magnetometers to archeology began with Aitken (1958) and a series of articles published in the journal, *Archaeometry*. Black and Johnston (1962) report on a magnetometer survey at the Angel site, a large Middle Mississippian village. Breiner and MacNaughton (1965) explain how magnetometers work and discuss the application of these instruments to land and marine archeology. Linington (1966) presents a discussion of magnetic theory and archeological interpretation of magneto-

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meter data. Pioneering work in the positioning control for magnetometer surveys in underwater archeology was done by Clausen (1966) in Florida. The survey of the Olmec site of San Lorenzo is a classic example of the use of the magnetometer to guide excavations on land (Breiner and Coe, 1972). Perhaps the most comprehensive articles on the archeological application of magnetometers are by Tite (1972) and Breiner (1973).

The site chosen for a magnetometer survey was a small Jornada Mogollon (Lehmer, 1948) village located thirty miles northeast of El Paso, Texas. Test excavations in the fall of 1972 revealed 3 shallow pithouses (Kegley, in preparation). These houses and additional ones located since the first field season were identified in order of discovery, House 1, House 2, etc. Three house locations are shown in Figure 1, which shows only a portion of the total excavated area.

Like other similar sites in the area, the village is blanketed with midden soil, seldom deeper than 15 cm. One radiocarbon date of A. D. 1150 $\pm$ 50 (TX 1735) is in general agreement with cross dating of ceramics. Although pit houses could be located by conventional methods such as trenching, there were no suface indications to aid in locating the structures. This, then, led to the exploration of remote sensing techniques.

The instrument utilized for this survey was a Varian Proton Magnetometer (M-50) with the sensor mounted on a 1.38m aluminum pole. A magnetometer senses distortions in the earth's magnetic field caused by the presence of ferrous compounds. Initially, it was not certain that the instrument would assist in delineating cultural features at this site. Local geology could be expected to produce a masking effect since the site is located about 100m from a massive outcrop of syenite porphyry called the Hueco Tanks. This, however, did not prove to be a problem. Test readings taken directly beside the outcrop recorded a 1500 gamma distortion, but successive stations moving away from the outcrop indicate that at a distance of 50m there was no noticeable interference.

No metallic iron artifacts could be expected in the prehistoric village, but the magnetometer is not limited to detecting anomalies caused by metallic iron. Iron in the form of the compounds hematite, maghemite and magnetite appears as a trace element in many clays. When the clay is fired as pottery, at temperatures above the Curie point of 580 C for magnetite and 650 C for hematite (Tite, 1972), the magnetic domains align themselves with the earth's magnetic field during cooling (each potsherd is like a small magnet). The remnant magnetization thus produced will cause a greater anomaly than an equal amount of randomly aligned magnetite in unfired clay. Therefore, concentrations of pottery could cause anomalies although the strength of the distortion would probably be small. This may in part be due to the randomized alignment of the remnant magnetization of the induvidual sherds. During the first season's excavation, pottery was found in significantly higher quantities in the fill above the floors of pit houses than about the structures. We believe that this is a

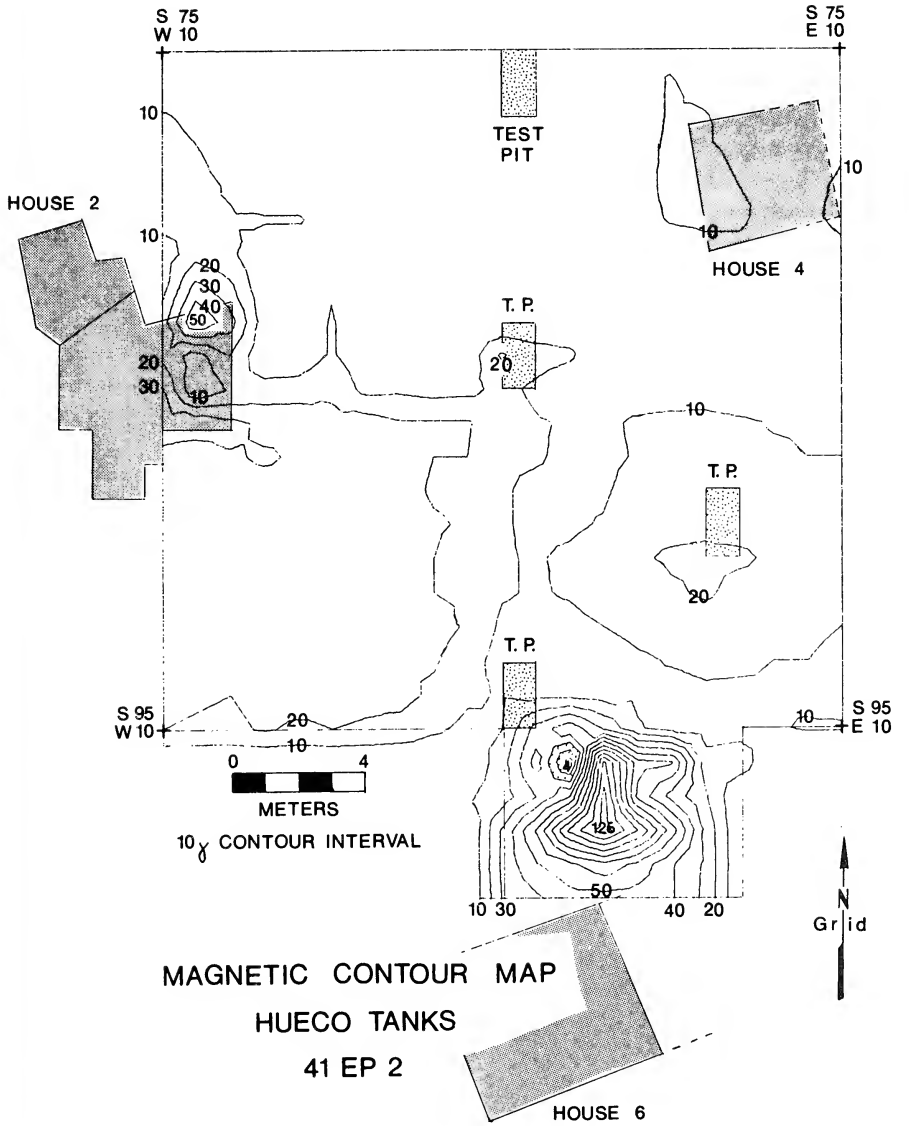


Figure 1. Computer drawn magnetic contour map showing a portion of 41EP2 with cultural features superimposed. Area delineated by line is the area of "in-site mode."

result of the abandoned pit houses having filled in with midden material. Burning of the houses could cause similar alignment of any traces of the ferri-magnetic compounds in the adobe used to line the pits. This could possibly cause a large anomaly (Tite, 1972).

Another cultural feature which could possible be detected by the magnetometer is the fired adobe hearth which is characteristic of these houses. One of the houses found in the first season was still exposed and since it had a hearth, the opportunity was provided for a test. The hearth was about 20 cm in diameter and caused only a 10 gamma anomaly and that only with the sensor directly above the hearth. At a distance of 1 m, the hearth did not register at all. This type of small hearth is not likely to be any help in this particular site in view of the amount of background noise caused by the overlying midden and local geological conditions. Experience at this site indicated that random $\pm$ 5 gamma deviations are not unusual in an area of thick midden deposits (this noise factor is well illustrated in the computer drawn perspective rendition of the magnetic data in Figure 2).

## METHOD AND RESULTS

The survey was divided into 2 phases consisting of an in-site mode and a search mode. The term "in-site" is used to refer to a tightly controlled systematic survey, with complete coverage of an area already established as part of a site (Clausen and Arnold, in preparation). Through this method is produced a magnetic contour map which can be used as a guide for the excavations. The search mode is of a more speculative nature, with the emphasis on prospecting for anomalies. The search mode is for initially locating an anomaly or a site or determining site boundaries.

At Hueco Tanks, the area between and adjacent to the known pit houses was selected for an in-site survey. Ten m. squares with corners corresponding to the original grid for the site were gridded with string at 1 m intervals. The small interval between the points of the data sample was necessary in view of the expected small magnitude of the anomalies. Two things soon became apparent: 1st, there would be no trouble picking up and defining anomalies at the site; and 2nd, setting up the control grid took much longer than acutually taking the data. The in-site mode was used to cover approximately 450 $m^2$ (Figure 1). Subsequent preparation of a contour map of the local variations in the earth's magnetic field showed 5 well defined anomalies.

Archeological testing in the fall of 1973 was begun after the magnetometer survey, by excavating test pits near the centers of magnetic anomalies or "peaks" plotted as contours (Figure 1). Results obtained from this excavation are considered significant. The following discussion is keyed to Figure 1. A test pit about 6 m. north of House 6 exposed a concentrated area of mixed burned rock and an ashy midden soil at a depth of 15 cm below the surface. House 6 has a contiguous room or rooms to the east which appear to have burned, raising the possibility that fire has been instrumental in producing the magnetic anomaly, which we tested, locating the north wall of the house. Burned structures and fire-cracked rock are rare in other portions of the site. Proceeding next to the area adjacent to House 2 (excavated in 1972), our excavations uncovered a

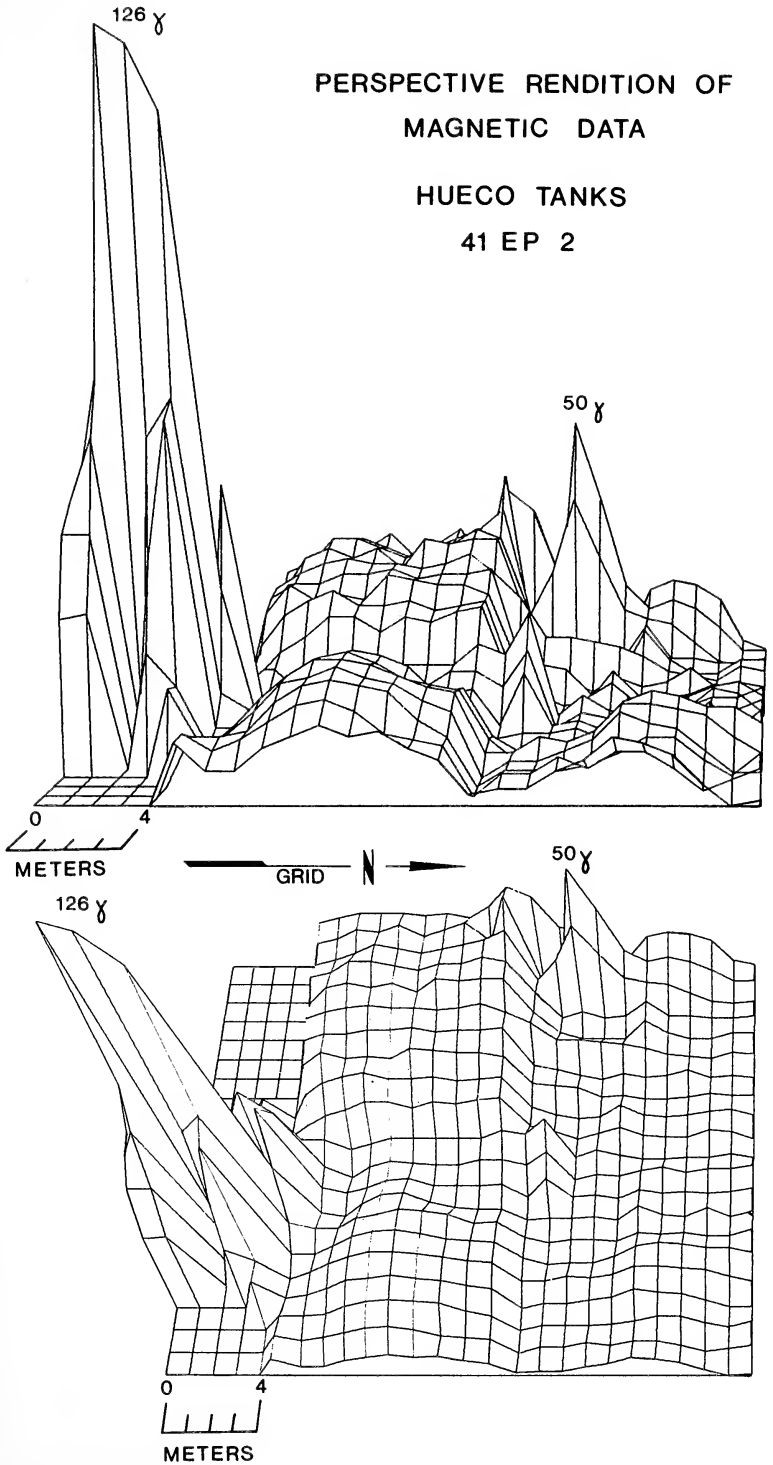


Figure 2. Computer drawn perspective views of magnetic data.

complex series of prepared superimposed pit house floors. A promising 50 gamma anomaly led us to test here. However, this pit house turned out to be quite similar to other pit houses at the site. No evidence of burning was noted, but a portion of the anomaly was no doubt caused by a 30 lb syenite porphyry metate found in a shallow depression in the house floor.

House 4 was located during test excavation of a 10 gamma anomaly. The situation is analogous to other pit houses at the site; the thin blanket-like cover of midden material thickens to a depth of about 1 m and extends from the ground surface to the floor of the house. In the pit fill there is a higher concentration of midden soil, melted adobe, potsherds, and lithic artifacts than in the surrounding midden.

A 20 gamma anomaly lies about 10 m south of House 4. Testing here produced no evidence of structures or features. This anomaly, however, may prove instructive since excavation again produced an unusually rich midden having a high yield of ceramic and lithic material.

The final anomaly appears in the center of Figure 1. It is spacially small and of 20 gammas intensity. The cause of this anomaly was found upon test excavation to be an iron reinforcement rod used the previous season to mark a grid corner.

As time was short, a limited search mode survey of a less precise and complete but more extensive nature was carried out. It was intended to sample the remainder of the site and perhaps define its limits. Results of this search mode survey are more difficult to evaluate, but it did result in locating several more anomalies and gave some feeling for the extent of the site. The noise gave way to relative quiet as the midden thinned out.

For the search mode survey, the magnetometer operator stood in the desired area. His position became the center of 2 circles of data samples, 8m and 4m in diameter. The samples on each circle were again spaced at 1m intervals. The centers of these circles were plotted. If an anomaly was detected, the center of the next sample was moved slightly to facilitate the delineation of its extent. The magnitude was recorded and the center of the anomaly was marked with a stake later to be shot in with alidade and plane table.

Prospecting in the search mode produced 6 anomalies varying in strength from 10 gammas to 80 gammas. Since time was not available for testing all 6 anomalies, 3 showing the greatest gamma deviation were chosen for trial excavations. Results are unclear since none of the anomalies checked produced cultural features nor offer easy explanation for the magnetic anomalies. The test pits lacked dense concentrations of artifacts or evidence of fire. Localized geological conditions can not be ruled out as causes of these anomalies.

## CONCLUSIONS

The in-site of survey has proven itself a most valuable guide for planning excavation. The search mode survey aided in delineating the extent of the site but

limited test excavations revealed no cultural features. Additional magnetometer work at Hueco Tanks would no doubt be fruitful. Further excavation would also provide useful information concerning the magnetic data already in hand. For example, one large area of magnetie disturbance in the 20 gammas range remains untested (Figure 1).

Another conclusion drawn from the survey data deals with the strength of various anomalies. In the area of the tightly controlled in-site survey, it seems that the magnitude of magnetic anomalies is less significant than the form or shape of these anomalies.

Breiner and Coe (1972) assert that no site is suited for magnetometer survey until such suitability is demonstrated. This survey, nevertheless, was successful in detecting anomalies representing cultural disturbances in a geological situation which at first glance would contra-indicate a successful magnetometer survey. However, we agree with them that someone with experience in magnetic surveying, preferably an archeologist, should be present to carry out the surveying and interpretation of the resulting data (*ibid.*).

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# A SODIUM IODIDE CENTRIFUGATION TECHNIQUE FOR ISOLATION OF NEWLY-REPLICATED DNA FROM RAT LIVER

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ABSTRACT

A simple procedure has been described for isolation of newly-replicated DNA from rat liver. Liver homogenates were lysed in 2% sodium dodecyl sulfate–0.1 M NaOH and centrifuged for 2.5 hr on alkaline sodium iodide step gradients. The procedure involved minimal handling of DNA and provided high resolution of newly-replicated DNA from bulk DNA.

## INTRODUCTION

Replication of mammalian DNA is a discontinuous process. Very short chains of approximately 100 nucleotides (thought to be analogous with the Okazaki fragments of bacteria) are formed and subsequently linked with longer, intermediate strands of DNA (Huberman and Horowitz, 1973). The intermediate strands join in turn to form bulk DNA (Hyodo, *et al.,* 1970).

Various analytical techniques, including autoradiography (Huberman and Riggs, 1968), sedimentation in sucrose gradients (Berger and Irvin, 1970; Huberman and Horowitz, 1973; Tsukada, *et al.,* 1968), and chromatography (Sato, *et al.,* 1972), have been utilized to characterize growing strands of DNA labelled with radioactive precursors. Techniques chosen for isolation and analysis of newly replicated DNA fragments should satisfy a number of criteria including minimal handling to avoid excessive shearing, adequate separation from bulk DNA, and sufficient yield for analysis. A rapid, simple procedure conforming to these criteria is described in the present report for isolation of newly replicated DNA from liver tissue. The procedure involves alkaline treatment of liver homogenates followed by centrifugation in alkaline sodium iodide step gradients.

## MATERIALS AND METHODS

### *Treatment of Animals*

Male albino rats from a colony maintained in this laboratory were used when they weighed 80–100 g. Synthesis of liver DNA was induced by removal of the

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median and left lateral lobes of the liver (Higgins and Anderson, 1931). [Methyl-$^{3}$H] Thymidine (17 Ci/mmole, purchased from Amersham-Searle) was administered via the tail vein at 19-22 hr after the partial hepatectomy.

*Preparation of Tissue Lysates and NaI Step Gradients*

Animals were killed by cervical dislocation. Approximately 1 g of liver was removed, homogenized in 4 ml of ice-cold 0.15 M NaCl with 4 strokes in a loose Dounce homogenizer, and filtered through 4 layers of cheesecloth. Filtered homogenate (0.5 ml) was pipetted into a tube containing an equal volume of 2% sodium dodecyl sulfate-0.1 M NaOH. Less than 1 min was required from the time the animal was killed to addition of homogenate to the alkaline solution. The tube was gently rolled to mix the contents and then left undisturbed for 60 min at room temperature.

Step gradients were formed in cellulose nitrate tubes fitting the Spinco SW 41 rotor by the successive addition of 2.8 ml layers of NaI-0.1 M NaOH solutions with densities of 1.5, 1.4, 1.3, and 1.2 g/cc, respectively. Lysed homogenates were then poured on top and the gradients centrifuged for 2.5 or 24 hr at 35,500 RPM and 18 C. Twelve 1 ml fractions were collected from the bottom of each gradient. The refractive index of each fraction was measured at room temperature with an Abbe refractometer and compared with alkaline NaI solutions of known densities.

*Determinations of Size of DNA Recovered from NaI Gradients*

NaI gradients were fractionated after centrifugation for 2.5 hr and bottom fractions containing bulk DNA were pooled. Fractions containing newly-replicated DNA collected from the middle of the gradients were pooled separately. The pooled fractions were dialyzed against 0.1 M NaOH-1 mM EDTA and then concentrated by dialysis against 2 M sucrose containing 0.1 M NaOH-1 mM EDTA. Aliquots (0.2 ml) of the concentrated solutions of newly-replicated and bulk DNA were layered on 5 ml gradients of 10-25% sucrose containing 0.9 M NaCl, 0.1 M NaOH, and 1 mM EDTA. Centrifugation was for 1.5 hr in a Spinco SW 50.1 rotor at 40,000 RPM and 18 C. $^{14}$C-Labelled $T_7$ DNA was used as a marker for estimation of the sedimentation coefficient of the newly-replicated DNA.

*Assay Procedures*

Half of each NaI gradient fraction was removed to 5 ml of ice-cold 10% trichloracetic acid (TCA). Acid-insoluble radioactivity was precipitated onto a 2.5 cm disc of Whatman No. 1 filter paper covered with 40 mg of 'Celite' (Johns-Manville), dried with ethanol and ether, and counted in a liquid scintillation solution containing 4 g of PPO and 0.05 g of POPOP/1 of toluene. DNA was precipitated from the remaining half with cold TCA and measured colorimetrically by reaction with diphenylamine (Burton, 1955).

Ten drop fractions were collected from the bottom of the alkaline sucrose gradients and adjusted to 1 ml with water. Absorbance was measured at 260 mμ and radioactivity was counted in a solution of toluene and Triton X-100.

## RESULTS

### *Centrifugation of Purified Nuclear DNA and Crude Homogenate*

Isolation of newly replicated DNA by NaI centrifugation of purified DNA was compared with results obtained when lysed whole homogenate was applied to the NaI gradients. Liver was removed from one rat 2 min after injection of 100 μCi of $^3$H-thymidine and nuclei were isolated as described previously (Brown, *et al.*, 1970). DNA, purified from the nuclei by the procedure of Zamenhof (1957), was redissolved in 0.15 M NaCl. Aliquots (0.5 ml) of the purified DNA were mixed with equal volumes of 2% sodium dodecyl sulfate-0.1 M NaOH and layered on two NaI gradients. Liver was removed from a second rat 2 min after injection of 100 μCi of $^3$H-thymidine, homogenized, lysed, and layered on two additional NaI gradients. One of the gradients with lysed homogenate and one with purified DNA were centrifuged for 2.5 hr. The other two gradients were centrifuged for 24 hr.

Results presented in Figure 1 suggest that physical manipulation of samples during preparation affected resolution of newly-replicated DNA from bulk DNA. The bulk of the DNA from lysed homogenate (samples subjected to minimal handling) sedimented close to the bottom of the gradients during the 2.5 hr centrifugation and most of the acid-insoluble radioactivity remained near the top (Figure 1A). Preparation of purified DNA required more extensive handling and the bulk of the purified nuclear DNA sedimented less than halfway through the gradient during the 2.5 hr centrifugation allowing only partial resolution of radioactive DNA (Figure 1B). Centrifugation of samples for 24 hr sedimented radioactivity and bulk DNA to the same equilibrium density (Figures 1C and 1D). This suggest that the radioactive peak at the top of the gradient shown in Figure 1A is newly-replicated DNA.

### *Distribution of Protein in Gradient Fractions*

Results presented in Table 1 indicate that NaI centrifugation effectively separated cellular proteins from DNA. A liver sample was lysed and centrifuged for 2.5 hr in a NaI gradient. Protein was precipitated from each gradient

TABLE 1

Distribution (%) of Protein in Gradient Fractions

| Fraction Number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 0.1 | 0.4 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | 0.4 | 0.4 | 1.6 | 30.4 | 65.6 |

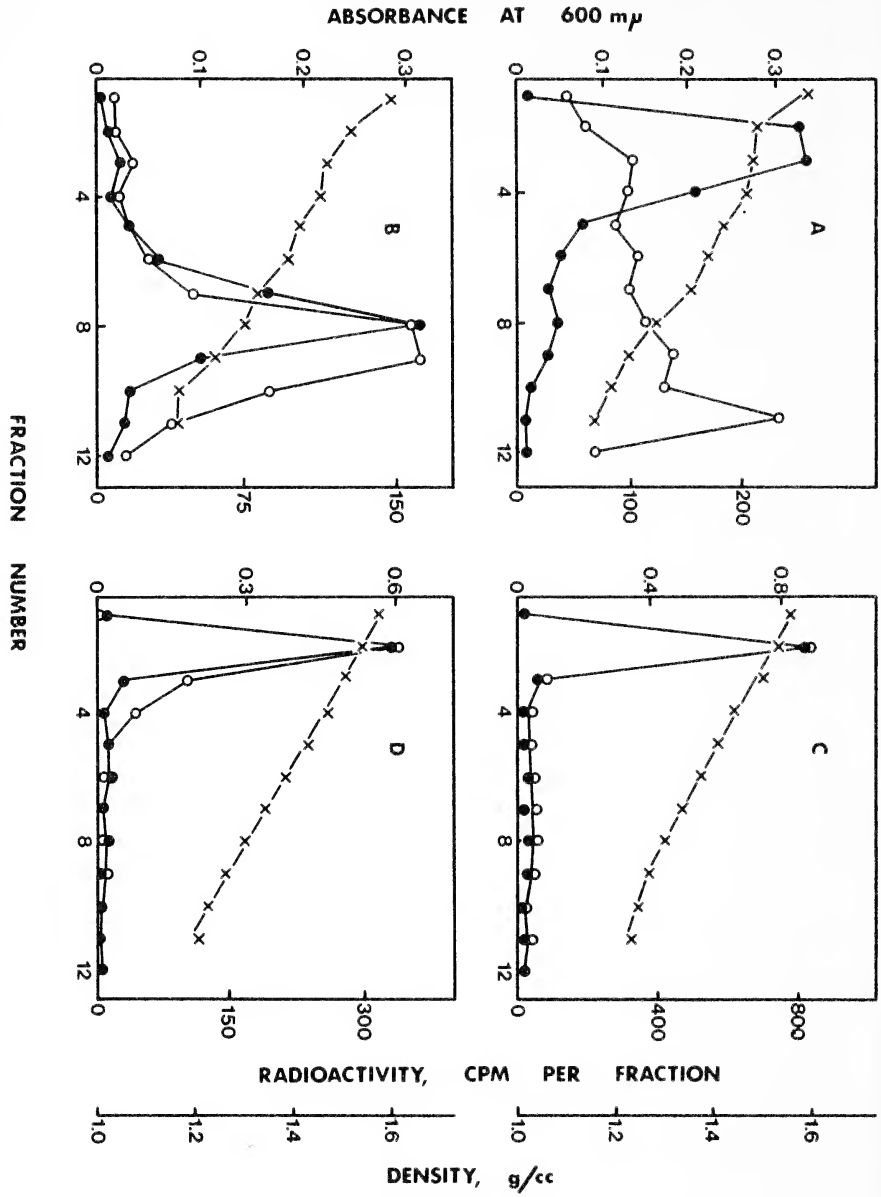


Figure 1. Comparison of NaI gradient sedimentation of lysed liver homogenate and purified liver DNA from rats pulse labelled with $^{3}$H-thymidine for 2 min. A. Lysed homogenate centrifuged for 2.5 hr. B. Purified DNA centrifuged for 2.5 hr. C. Lysed homogenate centrifuged for 24 hr. D. Purified DNA centrifuged for 24 hr. Radioactivity (o); absorbance (●); density (x).

fraction with cold 10% TCA and measured colorimetrically by the procedure of Lowry, *et al.*, (1951). As shown in the table, 96% of the protein from lysed homogenate was recovered at the top of the gradient (fractions 11 and 12). Very little protein was recovered with bulk DNA near the bottom (fractions 1-3) or in fractions containing most of the newly-replicated DNA (fractions 4-10).

*Effect of Pulse Duration on Sedimentation Profiles*

Sedimentation profiles shown in Figure 2 indicate that as the pulse duration increased, acid insoluble radioactivity shifted to bulk DNA. Liver samples were removed from 4 rats labelled with 10-100 $\mu$Ci of $^3$H-thymidine for durations of 2 to 60 min, lysed, and centrifuged for 2.5 hr in NaI gradients. Newly-replicated DNA from the animal pulse-labelled for 2 min with 100 $\mu$Ci was recovered in the top fractions of the gradient (Figure 2A). Pulse labelling for 5 min with 50 $\mu$Ci resulted in a broad peak of radioactivity in the middle of the gradient (Figure 2B). Removal of liver 10 min after injection of label (30 $\mu$Ci) resulted in a higher peak of radioactivity in bulk DNA than in the middle fractions (Figure 2C). And, finally, most of the acid-insoluble radioactivity was in bulk DNA in the sample from the rat pulse-labelled for 60 min with 10 $\mu$Ci. Similar profiles were obtained with preparations centrifuged for 2.5 hr at 41,500 RPM in a Spinco SW 50.1 rotor.

*Alkaline Sucrose Gradient Analysis of Products Recovered from a NaI Gradient*

Bulk and newly-replicated DNA prepared by centrifugation of lysed liver homogenate in a NaI gradient were analyzed by sedimentation in alkaline sucrose gradients. As shown in Figure 3, radioactive, newly-replicated DNA recovered from the middle of the NaI gradient was smaller than bulk DNA recovered from the bottom of the same gradient. To obtain these results liver was removed from one rat 5 min after injection of 50 $\mu$Ci of $^3$H-thymidine, lysed, and centrifuged for 2.5 hr in NaI. The NaI gradient was fractionated and fractions 1 and 2, containing bulk DNA, were combined. Fractions 6-8, containing newly-replicated, radioactive DNA, were pooled separately. Samples of bulk and newly-replicated DNA and $T_7$ DNA were then sedimented in separate tubes containing alkaline sucrose gradients. Bulk DNA sedimented close to the bottom of the first sucrose gradient. Newly-replicated DNA, however, sedimented less than halfway through the second sucrose gradient indicating that it was much smaller than the bulk DNA. Comparison of the position of the peak of radioactivity (fraction 14) with the position of the $T_7$ DNA marker (identified by the arrow) sedimented in an identical, third gradient yielded an estimated sedimentation coefficient (S) of 49 for the newly-replicated DNA. This estimate was based upon a published S value of 37.2 for $T_7$ DNA in alkaline solution (Studier, 1965).

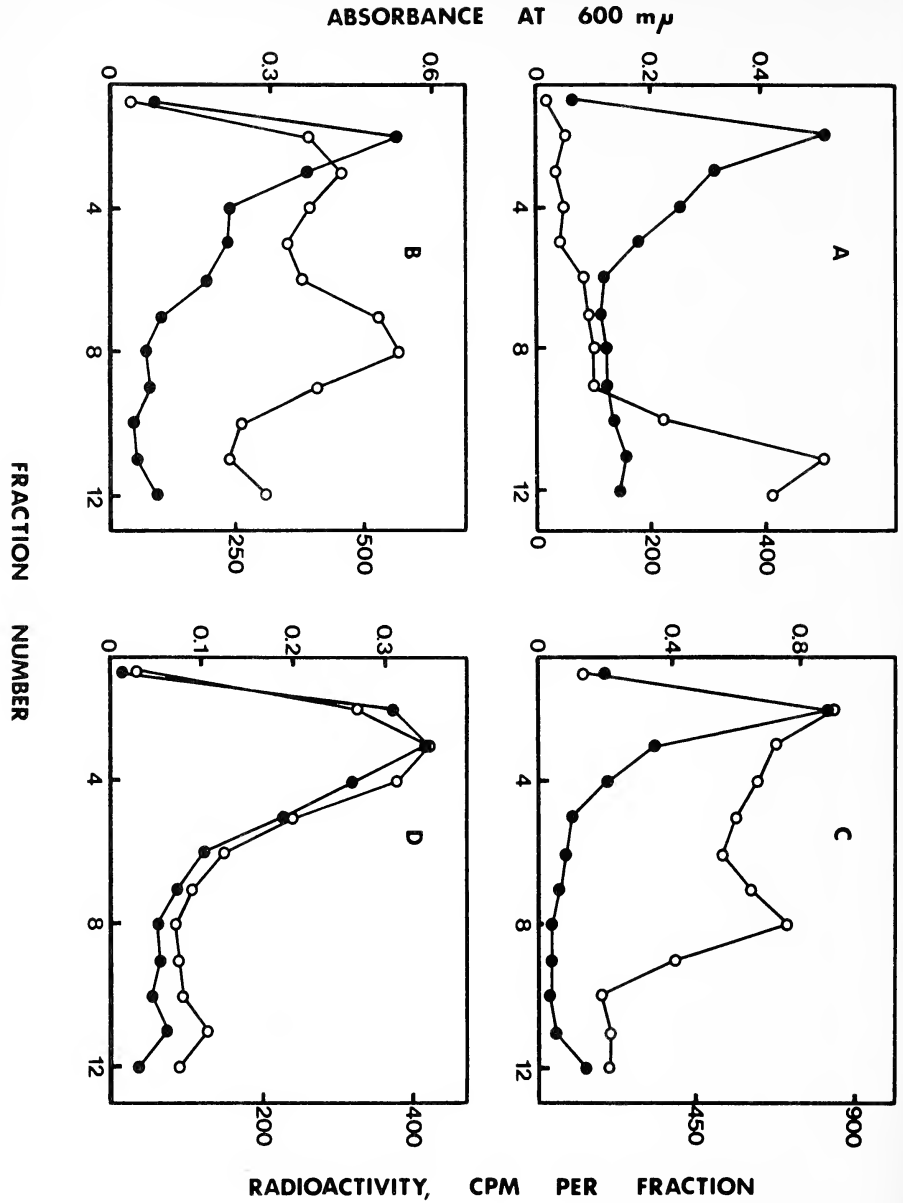


Figure 2. NaI gradient sedimentation of lysed liver homogenates from animals pulse-labelled with $^{3}$H-thymidine for: (A) 2 min; (B) 5 min; (C) 10 min; or (D) 60 min. Sedimentation was from right to left. Radioactivity (o); absorbance (●).

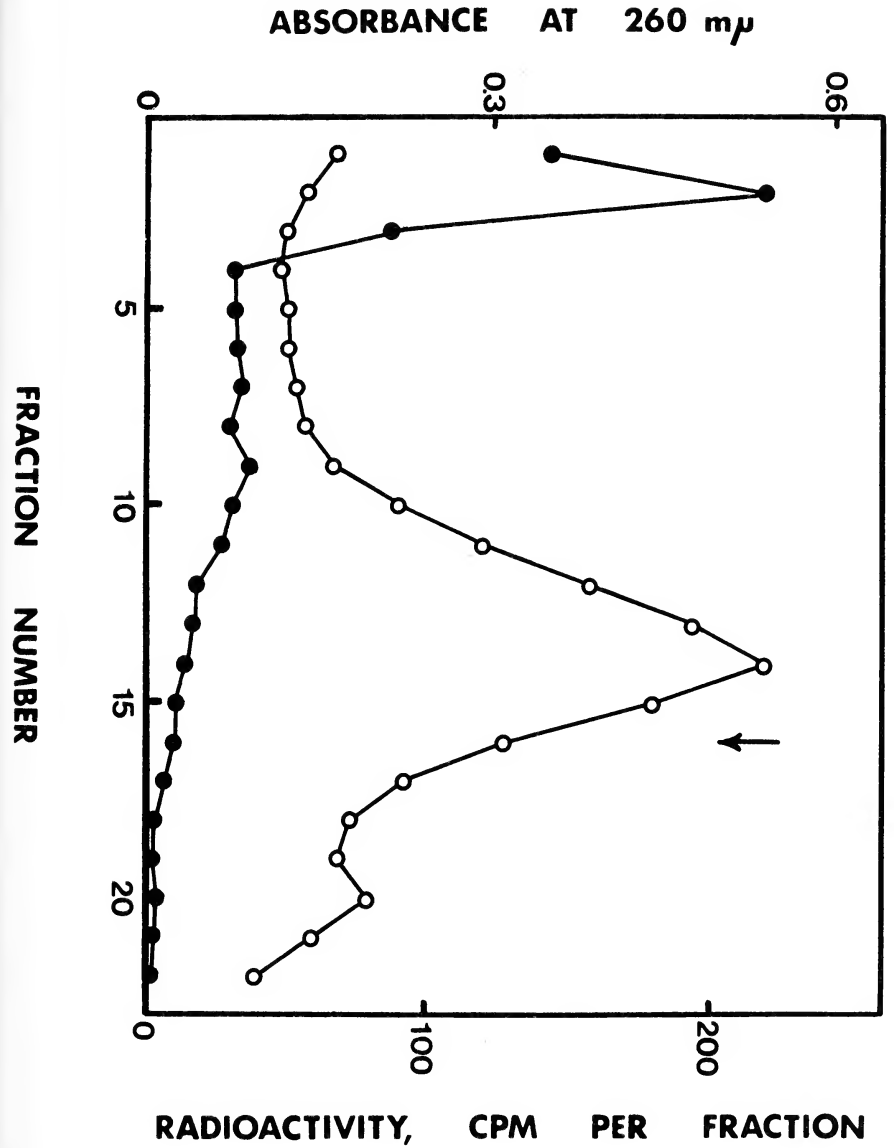


Figure 3. Alkaline sucrose gradient analysis of DNA's recovered from a NaI gradient. Sedimentation profiles of newly-replicated DNA and bulk DNA centrifuged in separate alkaline sucrose gradients (10–25%) are superimposed. The arrow represents the position of $^{14}C$-labelled $T_7$ DNA centrifuged in an identical alkaline sucrose gradient. Sedimentation was from right to left. Radioactivity (o); absorbance (●).

## DISCUSSION

The alkaline treatment of liver tissue and centrifugation of crude lysates on sodium iodide step gradients described in this paper provided good resolution of newly-replicated DNA from bulk DNA. Two factors appear to have contributed to separation of the DNA's. First, it was important to minimize handling of samples to reduce breakage of DNA. Shearing stresses were minimized by gentle lysis of liver tissue in alkaline solution. A similar alkaline lysis treatment was used by Lett *et al.*, (1967) for isolation of high molecular weight DNA from cultured cells. Bulk DNA from liver lysates sedimented to the bottom of the NaI gradients. When the bulk DNA was then recovered it was still highly viscous and sedimented more rapidly through alkaline sucrose gradients than did the newly-replicated DNA. Bulk DNA from the purified nuclear DNA sample, however, sedimented less than halfway through the NaI gradient. Separation from newly-replicated DNA was thus incomplete. This suggests that bulk DNA may have been sheared during purification to fragments not much greater in length than newly-replicated DNA.

Secondly, NaI step gradients were quite effective in separating newly-replicated DNA from bulk DNA if centrifugation was for 2.5 hr. Long durations of centrifugation sedimented DNA (both new DNA and bulk DNA) to its equilibrium density. Separation of the DNA's seemed to be a function of polynucleotide chain length as evidenced by the results of the sucrose gradient analysis of DNA recovered from a NaI gradient.

The NaI step gradients were developed as an alternative to conventional alkaline sucrose gradients. This investigator was not successful with centrifugation of crude lysates in sucrose gradients. The relatively dense lysates tended to partially sink when poured onto sucrose gradients, but layered very well on NaI as demonstrated by the fact that protein did not sediment appreciably into the NaI gradients.

The shift of acid-insoluble radioactivity from the top of the NaI gradient to the bottom with increased durations of $^{3}$H-thymidine incorporation appears to reflect growth of new strands of DNA and is consistent with the results obtained by Huberman and Horowitz (1973). These investigators observed progressive lengthening of radioactive DNA chains from short to intermediate and finally to bulk size as the length of the labelling period increased. The average size estimated (49S) for fragments of newly-replicated liver DNA recovered from the NaI gradients is within the size range observed from growing, intermediate strands of DNA in cultured cells (Huberman and Horowitz, 1973; Hyodo, *et al.*, 1970).

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# BARRIERS TO INTERNAL ROTATION IN 2-BROMOPROPENE AND 2-IODOPROPENE

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ABSTRACT

The 3-fold and 6-fold barriers to internal rotation of the methyl group were calculated from torsional frequencies to be 2640 and -106 cal/mole for 2-bromopropene and 2580 and -75 cal/mole for 2-iodopropene.

## INTRODUCTION

Vibrational spectra of 2-bromopropene have been published by Meyer and Gunthard (1967), and Meyer, *et al.*, (1969) have published the infrared spectrum of 2-iodopropene. The potential energy barrier hindering internal rotation of the methyl group in 2-bromopropene has been determined by microwave spectroscopy by Benz, *et al.*, (1966). The far infrared vapor and liquid-state spectra of 2-bromopropene had previously been determined by one of the present authors. Observation of the $0 \rightarrow 1$ and $1 \rightarrow 2$ torsional frequencies for both 2-bromopropene and 2-iodopropene has allowed calculation of not only the 3-fold barrier to internal rotation, but also the 6-fold barrier.

## EXPERIMENTAL

Far infrared spectra were obtained for 2-bromopropene with a Perkin-Elmer model 301 spectrophotometer, located at the Bartlesville Energy Research Center, through the courtesy of Dr. D. W. Scott. The sample of 2-bromopropene was obtained from Pfaltz and Bauer, and was used without further purification. No impurities were detected by gas chromatography.

### *Barriers to Internal Rotation*

The observation of both $0 \rightarrow 1$ and $1 \rightarrow 2$ torsional bands allows the determination of both $V_3$ and $V_6$ in the potential energy expression

$$V(\phi) = \tfrac{1}{2}V_3(1 - \cos 3\phi) + \tfrac{1}{2}V_6(1 - \cos 6\phi) + - - -$$

by the procedure outlined by Fateley and Miller (1963).

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The F value for 2-bromopropene was taken from Benz, *et al.*, (1966). The 3-fold barrier height is given by

$$V_3 = \frac{9}{4} F s$$

and s is obtained from tables of solutions for the Mathieu equation. The value of $V_3$ for 2-bromopropene is seen from Table 1 to be 923 $cm^{-1}$ or 2640 cal/mole, and $V_6/V_3$ = -0.04.

TABLE 1

Methyl Torsional Barrier Determination Summary.

| Compound | $v'' \rightarrow v'$ | $\omega$[a] ($cm^{-1}$) | F ($cm^{-1}$) | S | $V_3(cm^{-1})$ when $V_6/V_3$ is: 0.00 | -0.01 | -0.02 | -0.03 | -0.04 |
|---|---|---|---|---|---|---|---|---|---|
| $CH_3CBr=CH_2$ | $0 \rightarrow 1$ | 196.0 | 5.60 | 69.12 | 871 | 883 | 896 | 910 | 923 |
| | $1 \rightarrow 2$ | 183.6 | 5.60 | 70.89 | 893 | 901 | 908 | 915 | 923 |
| $CH_3CI=CH_2$ | $0 \rightarrow 1$ | 195.4 | 5.60 | 68.73 | 866 | 878 | 891 | 904 | 918 |
| | $1 \rightarrow 2$ | 182.2 | 5.60 | 69.99 | 882 | 889 | 896 | 903 | 910 |

[a]Observed frequencies are from this work for 2-bromopropene and from Meyer, *et al.*, (1969) for 2-iodopropene.

In order to justify the negative sign of $V_6$, it must be shown that the accuracy of the torsional frequencies is high enough that a negative $V_6$ is obtained for the complete range of uncertainty in the frequency measurement. Meyer and Gunthard (1967) determined the wavenumber of the $0 \rightarrow 1$ transition to be 195.7 $cm^{-1}$, with an uncertainty of 0.5 $cm^{-1}$, and they report the wavenumber of the $1 \rightarrow 2$ transition as 183 $cm^{-1}$, with an uncertainty of 1 $cm^{-1}$. Our values of 196.0 and 183.6 $cm^{-1}$ should be accurate to within these same uncertainties. Therefore, the limits of these 2 transitions should be 182.6 to 184.0 and 195.5 to 196.2 $cm^{-1}$.

The combination 196.2 and 182.6 $cm^{-1}$ (the high and low extremes, respectively, of the 2 frequencies) gives $V_3$ = 902 $cm^{-1}$, with $V_6/V_3$ = -0.022. while the other combination of extremes, 195.5 and 184.0 $cm^{-1}$, gives $V_3$ = 940 $cm^{-1}$ and $V_6/V_3$ = -0.056. Therefore, the maximum uncertainties are approximately 20 $cm^{-1}$ for $V_3$ and 0.017 for the $V_6/V_3$ ratio, based only on the accuracy of the torsional frequencies. The uncertainty in the value of F adds to the uncertainties in $V_3$ and $V_6$, but Hunziker and Gunthard (1965) point out that this is less than the uncertainty due to the frequency error. The value of $V_3$ should be accurate to within 100 cal, and our value, therefore, overlaps the microwave-determined value.

Use of the torsional wavenumbers reported by Meyer and Gunthard (1967), namely 195.7 and 183 $cm^{-1}$, yields $V_3$ = 2610 cal/mole and $V_6/V_3$ = -0.045.

The potential barrier hindering internal rotation of the methyl group in 2-iodopropene has not been determined by microwave spectroscopy, and structural data have not been obtained. Therefore, the F value for the internal rotation was taken

to be 5.60 $cm^{-1}$, which is the same as for 2-bromopropene. The values for 2-chloropropene, 5.62 $cm^{-1}$ (Unland, *et al.*, 1965), and 2-fluoropropene, 5.62 $cm^{-1}$ (Fateley and Miller, 1963), are almost the same.

Meyer, *et al.*, (1969) have determined the vapor-state infrared spectrum and they observed the $0 \rightarrow 1$ transition of the methyl torsion at $195.4 \pm 0.5$ $cm^{-1}$ and the $1 \rightarrow 2$ transition at $183.2 \pm 0.5$ $cm^{-1}$. The Fateley-Miller procedure was again used to obtain $V_3 = 902$ $cm^{-1}$, or 2580 cal/mole (see Table 1), and $V_6/V_3 = -0.029$. Use of the 0.5 $cm^{-1}$ uncertainties yields uncertainties of approximately 40 cal/mole in $V_3$ and 0.012 in the $V_6/V_3$ ratio. The uncertainty in the F value will increase the overall uncertainty in $V_3$ to approximately 70 cal/mole. The data used for both compounds is summarized in Table 1.

The methyl torsional barriers in the four 2-halopropenes are listed in Table 2. The value of $V_6$ for 2-bromopropene is 4% of $V_3$, and this is larger than any of the $V_6$ values for the compounds studied by Fateley and Miller (1963).

TABLE 2

Methyl Torsional Barriers in 2-Halopropenes.

| Compound | $V_3$ (cal/mole) | $V_6$ (cal/mole) | Method[a] | Reference |
|---|---|---|---|---|
| $CH_3CF=CH_2$ | 2440 | | MW | Peirce and O'Reilly (1959) |
| | 2340 | +4 | IR | Fateley and Miller (1963) |
| $CH_3CCl=CH_2$ | 2671 | | MW | Unland, *et al.*, (1965) |
| | 2645 | -49 | IR | Hunziker and Gunthard (1965) |
| $CH_3CBr=CH_2$ | 2695 | | MW | Benz, *et al.*, (1966) |
| | 2640 | -106 | IR | This work |
| $CH_3CI=CH_2$ | 2580 | -75 | IR | This work |

[a]MW=microwave, IR=infrared

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# THE POLAROGRAPHIC REDUCTION OF METAL IONS IN ACETONE AND ACETOPHENONE IN THE PRESENCE OF LITHIUM PERCHLORATE AND TETRAETHYLAMMONIUM PERCHLORATE

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ABSTRACT

The polarographic reduction behavior of certain metal ions in acetone and acetophenone in the presence of selected supporting electrolytes, lithium perchlorate and tetraethylammonium perchlorate, is observed.

## INTRODUCTION

The effect of supporting electrolytes on the polarographic reduction of metal ions has been examined by several investigators. Larson and Iwamoto (1960) reported that half-wave potential for the reduction of $Mn^{+2}$ in acetonitrile is more positive with 0.1 *M* tetraethylammonium perchlorate than with 0.1 *M* $Li^{+1}$, $Na^{+1}$ or $Mg^{+2}$ perchlorate as supporting electrolyte. They also noted similar behavior with tetraethylammonium perchlorate supporting electrolyte as compared with $Li^{+1}$ and $Mg^{+2}$ perchlorates as supporting electrolytes for the polarographic reduction of $Mn^{+2}$ in benzonitrile. In addition, Larson and Iwamoto (1960) also observed that with the exception of aluminum perchlorate, which is shifted in the positive direction, the perchlorates of $Ba^{+2}$, $Cr^{+3}$, $Fe^{+2}$, $Co^{+2}$, $Ni^{+2}$, $Zn^{+2}$ and $Cd^{+2}$ are reduced at more negative half-wave potentials in benzonitrile with 0.1 *M* $LiClO_4$ than with 0.1 *M* tetraethylammonium perchlorate as supporting electrolyte. Schaap, *et al.*, (1961) studied the polarographic reduction of $Tl^{+1}$, $Pb^{+2}$, and $Cd^{+2}$ with 0.1 *M* $NaNO_3$ in ethylenediamine and concluded that the shifts in the half-wave potentials were attributed to ion-pair formation between the reducible metal ion and anion of the inert electrolyte. Similarly, Schober and Gutman (1958, 1959, 1960, 1961) examined the polarographic behavior of a number of metal salts in ethylenediamine, and stated that the half-wave potentials of metal ions depended upon the supporting electrolyte and the anion of the reducible metal ion.

In an effort to obtain additional information on the effect of inert electrolytes on the polarographic behavior of metal ions, we have chosen to carry out 2 studies, the polarographic reduction of metal ions in acetone and acetophenone in the

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presence of lithium and tetraethylammonium perchlorates, and the effect of inert electrolytes on the formation constants of aquo-$Cu^{+2}$ complexes in acetone.

Acetone, dielectric constant 21, and acetophenone, dielectric constant 17, have been neglected as solvents for electro-chemical studies.

Only 4 polarographic studies have been reported for acetone as a solvent. Arthur and Lyons (1952) observed well-defined reduction waves for acid bromides and acid chlorides in acetone, but an incompletely developed reduction wave for $Cd^{+2}$ in acetone with LiCl as supporting electrolyte. Ozerov and Yakovleva (1956) obtained polarograms for the reduction of $Sr^{+2}$ and $Bi^{+3}$ nitrates, $Cu^{+2}$ and $Zn^{+2}$ chlorides, $CdI_2$ and CuBr in acetone solutions without supporting electrolyte and in acetone solutions containing 0.5 *M* $LiNO_3$ as supporting electrolyte. Migal, *et al.*, (1956) studied the polarography of HCl in acetone with LiCl as supporting electrolyte. They observed that the diffusion current constant of HCl increased with increasing concentration of HCl. Coetzee and Siao (1963) investigated the polarographic behavior of the rare earth elements in acetone with 0.1 *M* tetraethylammonium perchlorate supporting electrolyte. These investigators concluded that the reduction of the +3 oxidation state of the rare earths led to $H_2$ evolution rather than amalgam formation, and that acetone is a differentiating solvent for the polarographic reduction of $HClO_4$ and $H_2SO_4$.

## EXPERIMENTAL

The organic solvents were purified by treatment with appropriate reagents and fractional distillation (Weissburger and Proskauer, 1955; Vogel, 1956). Reagent-grade acetone was treated with $KMnO_4$, dried over anhydrous $K_2CO_3$ and distilled from $P_2O_5$. Highest available purity acetophenone was dried over anhydrous $CaCl_2$ and fractionally distilled.

Tetraethylammonium perchlorate was prepared from Eastman tetraethylammonium bromide as previously described (Kolthoff and Coetzee, 1957). All the metal perchlorates were obtained from G. F. Smith Chemical Company and were dried in a vacuum over 60 C for 48 hrs. The residual water content of the perchlorate salts was not determined; however, the colors of the dried meterials in comparison to the corresponding hydrated salts indicated that the drying operation reduced the amount of water in the salts. The total water content of the acetone solutions containing supporting electrolyte and $Cu(ClO_4)_2$ was determined with a modified Karl Fischer reagent (Mitchell and Smith, 1958). The water content of the acetophenone solutions was approximately 0.02 *M*.

Current-voltage curves were obtained with a Kelly, *et al.*, (1959) and Larson and Iwamoto (1962) controlled potential polarograph. All solutions were examined at 25 C in a thermostatically controlled bath. An H-type cell, with the 2 compartments separated by a single glass disc, was used. The dropping mercury electrode was placed in one side arm, and a saturated colomel electrode along with a platinum-foil electrode to function as the auxilliary electrode in a 3-electrode system was placed in the other.

## RESULTS AND DISCUSSION

### *Effect of Supporting Electrolyte on Half-Wave Potentials*

Table 1 lists the half-wave potentials for the reduction of $Na^{+1}$, $Cd^{+2}$, $Mn^{+2}$, $Zn^{+2}$, $Cr^{+3}$, $Cr^{+2}$, $Ni^{+2}$, $Pb^{+2}$, $Cu^{+2}$, $Cu^{+1}$ and $Mg^{+2}$ ions in acetone and acetophenone in the presence of 0.1 *M* $LiClO_4$. The behavior observed in acetone for $Na^{+1}$, $Mg^{+2}$, $Cd^{+2}$, $Cu^{+2}$ and $Cu^{+1}$ in the presence of both supporting electrolytes is similar to the behavior observed by Coetzee and Siao (1963).

TABLE 1

Half-Wave Potentials of Metal Ions in Acetone and Acetophenone.

| Metal[b] | Acetone | | | |
|---|---|---|---|---|
| | $E_{1/2}$[a] | Slope[d] | $E_{1/2}$[a] ($Et_4NClO_4$) | Slope[d] |
| $Na^{+1}$ | -- | -- | -1.90 | 0.062 |
| $Cd^{+2}$ | -0.20 | 0.033 | -0.21 | 0.036 |
| $Mn^{+2}$ | -1.11 | 0.035 | -1.12 | 0.039 |
| $Zn^{+2}$ | -0.57 | 0.037 | -0.61 | 0.031 |
| $Ni^{+2}$ | -0.54 | 0.100 | -0.57 | 0.105 |
| $Pb^{+2}$ | -0.12 | 0.034 | -0.12 | 0.030 |
| $Cu^{+2}/Cu^{+1}$ | +0.58 | 0.068 | +0.55 | 0.058 |
| $Cu^{+1}/Cu^{0}$ | +0.33 | 0.065 | +0.38 | 0.650 |
| $Mg^{+2}$ | -- | -- | -1.73 | 0.080 |
| $Sr^{+2}$ | -- | -- | -1.93 | 0.060 |
| $Cr^{+3}/Cr^{+2}$ | -- | -- | -- | -- |
| $Cr^{+2}/Cr^{0}$ | -- | -- | -- | -- |
| Metal[b] | Acetophenone | | | |
| | $E_{1/2}$[a] ($LiClO_4$) | Slope[d] | $E_{1/2}$[a] ($Et_4NClO_4$) | Slope[d] |
| $Na^{+1}$ | -- | -- | -- | -- |
| $Cd^{+2}$ | -0.17 | 0.028 | -0.16 | 0.028 |
| $Mn^{+2}$ | -1.05 | 0.049 | -1.05 | 0.047 |
| $Zn^{+2}$ | -0.55 | 0.037 | -0.53 | 0.040 |
| $Ni^{+2}$ | -0.51 | 0.081 | -0.47 | 0.105 |
| $Pb^{+2}$ | -0.08 | 0.028 | -0.03 | 0.030 |
| $Cu^{+2}/Cu^{+1}$ | -- | -- | -- | -- |
| $Cu^{+1}/Cu^{0}$ | -- | -- | -- | -- |
| $Mg^{+2}$ | -- | -- | -- | -- |
| $Sr^{+2}$ | -- | -- | -- | -- |
| $Cr^{+3}/Cr^{+2}$ | -0.50 | 0.059 | -0.46 | 0.058 |
| $Cr^{+2}/Cr^{0}$ | -0.93 | 0.081 | -0.97 | c |

[a] Versus S.C.E.
[b] 0.1 mmol concentration
[c] Drawn out wave.
[d] 0.059/n

It is also seen from the results presented in Table 1 that the waves of $Mn^{+2}$, $Zn^{+2}$, $Sr^{+2}$, and $Pb^{+2}$ are reversible. $Ni^{+2}$ gives one well-defined diffusion plateau but is somewhat drawn out.

The results obtained with solutions of the perchlorates of $Cd^{+2}$, $Mn^{+2}$, $Zn^{+2}$, $Ni^{+2}$, $Pb^{+2}$, $Cr^{+3}$, and $Cr^{+2}$ in acetophenone in the presence of both supporting electrolytes is presented in Table 1. The observed half-wave potentials are in the same order as those in acetone. $Cd^{+2}$, $Zn^{+2}$, and $Pb^{+2}$ give well-defined waves corresponding to a 2-electron reduction. $Mn^{+2}$ and $Ni^{+2}$ give well-defined diffusion plateaus, but are somewhat drawn out.

$Cr^{+3}$ gives a well-defined first wave corresponding to a 1-electron reduction, and a drawn out wave corresponding to the reduction of $Cr^{+2}$ to the metal. Acetophenone is reduced at a potential of -1.4V *vs* S.C.E. The investigation of the polarographic behavior of any metal ion which is reduced at potentials more negative than 1.4V *vs* S.C.E., therefore, is not possible.

In the presence of 0.1 *M* $LiClO_4$ the average $E_{1/2}$ ($E_{1/2\ acetone}$-$E_{1/2\ acetophenone}$), for the reduction of the metal ions is approximately -0.03V, whereas, for 0.1 *M* tetraethylammonium perchlorate, the average $E_{1/2}$ is approximately -0.8V. This suggests that the solvated metal ion undergoing reduction is acetone and acetophenone is more nearly the same species when 0.1 *M* $LiClO_4$ is used as the supporting electrolyte.

*Effect of Supporting Electrolyte on Formation Constants*

Figure 1 contains the experimental data, in the form of the half-wave potential for the reduction of $Cu^{+2}$ to $Cu^{+1}$ *vs* the negative logarithm of the total water concentration, for the polarographic study of the interaction of small amounts of water in acetone with $Cu^{+2}$ in 0.1 *M* $LiClO_4$ and 0.1 *M* tetraethylammonium perchlorate supporting electrolyte solutions. Curve A shows the experimentally observed dependency of the half wave potential for the $Cu^{+2}/Cu^{+1}$ couple on the total water concentration with $LiClO_4$ as supporting electrolyte, and Curve B shows the experimentally observed dependency of the half-wave potential for the $Cu^{+2}/Cu^{+1}$ couple on the total water concentration with tetraethylammonium perchlorate as supporting electrolyte.

Over the range of water concentrations studied, there was no change in the liquid-junction potential with either supporting electrolyte. [$E_{1/2\ Et_4NClO_4}$ *vs* S.C.E. in 0.01 *M* to 0.5 *M* water +0.942V, and $E_{1/2\ LiClO_4}$ *vs* S.C.E. in 0.01 *M* to 0.5 *M* water = +0.940V for the oxidation of tris-4,7-dimethyl-1,10-phenanthroline $Fe(ClO_4)_2$]. There appears to be only a slight change in the solvation energy of $Cu^{+1}$ ion over the range of water concentration studied, as evidenced by a change of only 5mV in the half-wave potential of the $Cu^{+1}$/Cu(Hg) couple. Consequently, the change in half-wave potential for the $Cu^{+2}/Cu^{+1}$ wave with water concentration is due essentially entirely to the complexation of $Cu^{+2}$ ions by water molecules, and due not to the interaction of water with $Cu^{+1}$, nor to changes in liquid-junction potential.

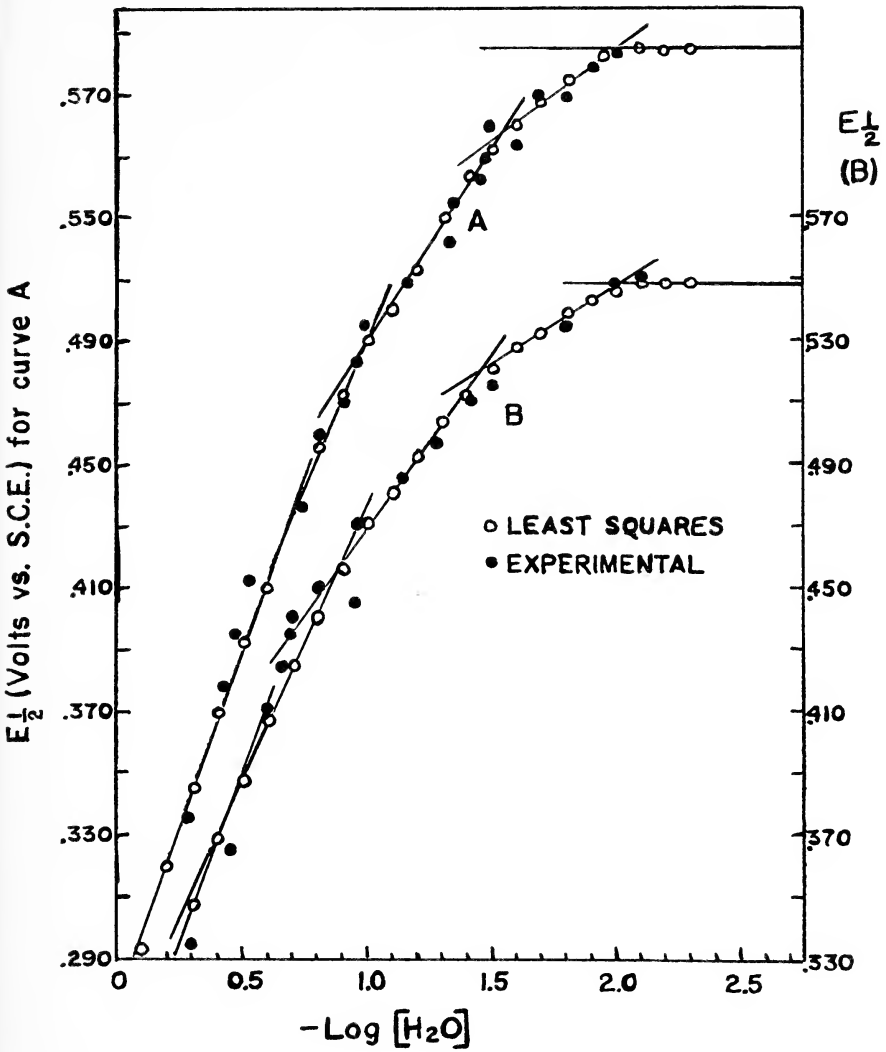


Figure 1. Half-wave potential, $E_{1/2}$ for the $Cu^{+2}$, $Cu^{+1}$ wave in acetone as a function of -log $[H_2O]$: Curve A 0.1 *M* $LiClO_4$ supporting electrolyte; Curve B 0.1 *M* $(C_2H_5)_4NClO_4$, supporting electrolyte.

The graphically evaluated formation constants from the 2 sets of data are listed in Table 2.

Because the graphically evaluated constants are at best good approximations of the dissociation constants, better values were calculated through the method of successive approximations. The average calculated values, using Equation 1, for log k are presented in Table 3.

TABLE 2

Graphically Evaluated log Formation Constants for Aquo-$Cu^{+2}$ Complexes in Acetone.

| 0.1 *M* $LiClO_4$ | 0.1 *M* $Et_4NClO_4$ |
|---|---|
| $\log k_1 = 2.00$ | $\log k_1 = 1.93$ |
| $\log k_2 = 1.52$ | $\log k_2 = 1.37$ |
| $\log k_3 = 1.05$ | $\log k_3 = 0.84$ |
| $\log k_4 = 0.64$ | $\log k_4 = 0.33$ |

$$E_{1/2} = E^\circ - 0.059 \log \left[1 + \frac{[H_2O]}{K_1} + \ldots \frac{[H_2O]^n}{K_1 \ldots K_n}\right] \quad (1)$$

where

$$K_1 = \frac{[Cu^{\#}]\ [H_2O]}{[Cu(H_2O)^{\#}]} = \frac{1}{K_1} \quad (2)$$

and

$$K_N = \frac{[Cu(H_2)_{N-1}{}^{\#}]\ [H_2O]}{[Cu(H_2O^{\#}{}_N]} = \frac{1}{K_n} \quad (3)$$

and

$$E^{\circ 1} = E^\circ + 0.059 \log \frac{K_{Cu^{+1}} f_{Cu^{+2}} f_{H_2O}}{K_{Cu^{+2}} f_{Cu^{+1}}} \quad (4)$$

TABLE 3

Average Values of pk log K Obtained with Equation 1 by the Method of Successive Approximations Aquo-$Cu^{+2}$ Complexes in Acetone.

| 0.1 *M* $LiClO_4$ | 0.1 *M* $Et_4NClO_4$ |
|---|---|
| $\log k_1 = 1.75$ | $\log k_1 = 1.73$ |
| $\log k_2 = 1.52$ | $\log k_2 = 1.27$ |
| $\log k_3 = 0.99$ | $\log k_3 = 0.80$ |
| $\log k_4 = 0.73$ | $\log k_4 = 0.66$ |

## SUMMARY

From the results of the data on the $Cu^{+2}/Cu^{+1}$, $Cu^{+1}/Cu^0$ reactions in the presence of 0.1 *M* $LiClO_4$ or 0.1 *M* tetraethylammonium perchlorate, the following inferences can be drawn.

1. Although $Li^{+1}$ ion has a higher affinity for water than tetraethylammonium ion, the formation constants for aquo-$Cu^{+2}$ complexes in acetone are very nearly alike in the presence of $LiClO_4$ and tetraethylammonium perchlorate.

2. Similarly, ion-ion interactions between $Li^{+1}$ and perchlorate and tetraethylammonium and perchlorate would be expected to affect the stability constants of aquo-$Cu^{+2}$ complexes in acetone containing these perchlorates. No such effect is observed.
3. Finally, it is of interest to note that Larson and Iwamoto (1962) have obtained similar results in their study of the formation constants of aquo-$Cu^{+2}$ complexes in nitromethane with and without added electrolyte. These investigators found that the average individual formation constants of the aquo-$Cu^{+2}$ complexes obtained by electrochemical means with 0.1 *M* tetraethylammonium perchlorate as supporting electrolyte agreed very well with the average individual formation constants obtained by spectrophotometric means without any added electrolyte.

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# EFFECTS OF SIMPLE SUGARS ON CERTAIN SERUM CONSTITUENTS AND ERYTHROCYTE OSMOTIC FRAGILITY IN RATS[1]

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ABSTRACT

Four groups of rats were maintained on isocaloric, isonitrogenous, chemically defined diets differing only in carbohydrate source, i.e., glucose, fructose, sucrose, and a combination of glucose and sucrose. The effects of such dietary alteration were examined with respect to the following: serum proteins, serum glycoproteins, serum haptoglobin, serum bilirubin, as well as erythrocyte osmotic fragility. The results of these studies indicate abnormal test values for those animals on the fructose diet.

## INTRODUCTION

In previous studies in this laboratory (unpublished data), we have observed that rats fed diets containing different amounts of sucrose and fats showed unusual serum glycoprotein profiles when compared with profiles reported by other workers (Cockerell and Beisel, 1973). The greatest percentage of protein-bound carbohydrates was found to migrate as $\alpha_2$ glycoproteins, regardless of the amount and type of sucrose or lipid offered in the diet. Considerable interest has been shown by other investigators in serum proteins, glycoproteins, and particularly haptoglobin, an $\alpha_2$ glycoprotein, in man and animals (Brus and Lewis, 1959; Deutsch and Goodloe, 1945; Fink, *et al.*, 1967; Krauss and Sarcione, 1963; Nyman, 1959; Pearce, *et al.*, 1964; Pigman, 1969; Purdom, *et al.*, 1972, 1973; Spiro, 1963). However, the influence of a chemically defined high carbohydrate diet on these parameters has not been reported.

In addition to the high $\alpha_2$ glycoprotein levels found in our previous studies, a considerable degree of erythrocyte hemolysis in rats maintained on diets containing a high sugar content has been observed (unpublished data; Purdom, *et al.*, 1972, 1973). Thus, under the above dietary conditions, there may exist a relationship between serum haptoglobin, the hemoglobin binding protein with the highest carbohydrate content of the $\alpha_2$ glycoproteins (Berk, *et al.*, 1969; Bieri and Evarts, 1975; Bieri and Poukka, 1970; Brus and Lewis, 1959; Fink, *et al.*, 1967; Grodsky,

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*et al.*, 1962; Kraus and Sarcione, 1963; Nyman, 1959; Ostrow, *et al.*, 1962, 1963; With, 1954), serum bilirubin, a metabolite of hemoglobin, and erythrocyte hemolysis.

The investigation reported herein was designed to determine the effects of feeding Sprague-Dawley male rats isocaloric, isonitrogenous chemically-defined diets containing a high percentage of glucose, fructose, and sucrose or a combination of equal amounts of glucose and sucrose as a sole source of carbohydrate. A moderate level of corn oil was chosen as the lipid component of diets. The influence of these diets was monitored for their effects on the following: serum proteins; serum glycoproteins; serum haptoglobin; serum bilirubin; and erythrocyte osmotic fragility.

## METHODS

Twenty male Sprague-Dawley rats were divided into 4 groups of approximately equal average weight. The average starting weight of the entire 20 animals was 152.8 gm with individual weights ranging from 136 to 164 gm. Each animal was kept in a stainless steel metabolic cage and fed daily (16-20 gm) of isocaloric, isonitrogenous chemically defined test diets (Table 1) for 28 days. Water was given *ad libitum*. All animals were weighed on the same day each week. The daily food offered to each animal was recorded and the uneaten food from each cage was collected and weighed to determine the amount actually consumed.

TABLE 1

Composition of Experimental Diets.[a]

| Constituents | Diet | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Amino acid mixture[b] | 139.95 | 139.95 | 139.95 | 139.95 |
| Alphacel (hydrolyzed) | 50.00 | 50.00 | 50.00 | 50.00 |
| Choline chloride | 2.00 | 2.00 | 2.00 | 2.00 |
| Salt mixture RH[b] | 40.00 | 40.00 | 40.00 | 40.00 |
| Vitamin mixture[c] | 5.00 | 5.00 | 5.00 | 5.00 |
| Corn oil (Mazola) | 101.05 | 101.05 | 101.05 | 101.05 |
| Cerelose (D-glucose)[d] | 662.00 | ---- | ---- | 331.00 |
| Fructose | ---- | 662.00 | ---- | ---- |
| Sucrose | ---- | ---- | 662.00 | 331.00 |

[a]Nitrogen content of all diets was 2.24%. All diets contained approximately 4.0 kcal/gm.
[b]Rogers and Harper (1965).
[c]Per kilogram vitamin mixture: Vitamin A, 900,000 I.U.; Vitamin D, 100,000 I.U.; alpha tocopherol, 5.0 gm; ascorbic acid, 45.0 gm; inositol, 5.0 gm; choline chloride, 75.0 gm; riboflavin, 1.0 gm; menadione, 2.25 gm; p-aminobenzoic acid, 5.0 gm; niacin, 4.5 gm; pyridoxine hydrochloride, 1.0 gm; thiamin hydrochloride, 1.0 gm; calcium pantothenate, 3.0 gm; biotin, 20 mg; folic acid, 90 mg; Vitamin $B_{12}$, 1.35 mg.
[d]Cerelose Corn Products Refining Co., Argo, Illinois.

At the termination of the experiment, all animals were fasted for 14 hr, anesthetized with absolute ether, and blood was drawn into sterile syringes following standard cardiac puncture. Approximately 2-3 cc of whole blood from each rat was transferred to separate vacutainers containing 0.04 ml of a 15% solution of EDTA (tripotassium salt) and 0.008 mg potassium sorbate and immediately refrigerated. These blood samples were employed in the studies of osmotic fragility. All other blood drawn from each rat was transferred into individual sterile test tubes and immersed in a 37 C water bath until firm and fully retracted clots formed. The clotted blood samples were centrifuged at full speed in a clinical centrifuge for 30 min. The serum was withdrawn and transferred to separate containers containing 0.04 ml of the above EDTA solution.

Total serum proteins were determined using a calibrated T/C refractometer.[1] Serum proteins were electrophoresed on Titan III cellulose acetate plates[2] and stained with Ponceau S according to a published procedure (Chin, 1970). Serum glycoproteins were electrophoresed on Titan III cellulose acetate plates in like manner and stained with Schiff's reagent (Kelsey, *et al.*, 1964). The resulting electrophoretograms were scanned in a densitometer[3] with a digital integrator.

Serum haptoglobin levels were determined by single radial immunodiffusion employing the Behring Diagnostics M-Partigen Haptoglobin Accupak™ Kit[4]. A standard curve was constructed from Behrings Protein Standard Serum B which is a stabilized pooled human serum consisting of 3 pre-diluted standards containing 68 mg%, 136 mg%, and 260 mg% haptoglobin respectively. The Mancini simplified end point methodology was then utilized for quantitation (Mancini, *et al.*, 1965).

The total serum bilirubin and erythrocyte osmotic fragility determinations were conducted according to the method of Davidson and Henry (1969).

Means and standard deviations are reported in each table to show the variability within the diets. Means were compared by TuKey's multiple range test at the 5% level of significance (Winer, 1971).

## RESULTS AND DISCUSSION

### *Growth Response*

The average weight gain per day of 5 animals maintained on the test diets (Table 1) was: Diet 1, 5.2 g; Diet 2, 4.6 g; Diet 3, 5.2 g; and Diet 4, 5.2 g. The overall total consumption of the food offered animals on Diets 1, 3, and 4 averaged 96, 91, and 94% respectively, while those animals on Diet 2 averaged 88% food consumption. Animal weight gain corresponds well with food consumption.

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[1] Model 10402, American Optical Corporation, Buffalo, New York.

[2] Helena Laboratories, Beaumont, Texas.

[3] Photovolt Model 542A.

[4] M-Partigen Haptoglobin Kit (No. 12-749-001), Behring Diagnostics, Sommerville, New Jersey.

*Serum Proteins*

The distribution of serum proteins obtained electrophoretically is presented in Table 2. Plasma volumes were approximately the same for all animals. The albumin, alpha$_1$, alpha$_2$, and gamma globulin fractions displayed no statistically significant changes with respect to the various diets. However, differences were noted among the following: total protein, beta globulins, and the albumin/globulin (A/G) ratio. The smallest total protein values were observed for animals on the glucose diet; the beta globulin fraction varied according to diet, with the least value observed for Diet 3 animals and the greatest for Diet 2 animals. Perhaps of significance is the fact that fructose-fed animals (Diet 2) had the lowest A/G ratio, 1.4, while those animals on the sucrose diet had the highest A/G ratio, 2.2.

TABLE 2

Effect of Experimental Diets on the Distribution of Serum Proteins.

| Serum Determination | Concentration of Serum Constituents (Gm %[a]) | | | |
|---|---|---|---|---|
| | Diet 1 (G)[b] | Diet 2 (F)[b] | Diet 3 (S)[b] | Diet 4 (G-S)[b] |
| Total protein | 5.5[c] ± 0.29 | 6.1[d] ± 0.30 | 6.0 ± 0.32 | 6.0[d] ± 0.27 |
| Albumin | 3.6 ± 0.32 | 3.6 ± 0.30 | 4.1 ± 0.18 | 3.7 ± 0.38 |
| Alpha$_1$ Globulin | 0.6 ± 0.02 | 0.8 ± 0.16 | 0.5 ± 0.17 | 0.8 ± 0.24 |
| Alpha$_2$ Globulin | 0.3 ± 0.13 | 0.5 ± 0.05 | 0.4 ± 0.20 | 0.4 ± 0.25 |
| Beta Globulin | 0.8 ± 0.17 | 1.0[d] ± 0.11 | 0.7[c] ± 0.23 | 0.9 ± 0.13 |
| Gamma Globulin | 0.2 ± 0.08 | 0.2 ± 0.09 | 0.3 ± 0.09 | 0.2 ± 0.12 |
| Total Globulin | 1.9 ± 0.25 | 2.5 ± 0.35 | 1.9 ± 0.42 | 2.3 ± 0.51 |
| A/G | 1.9 ± 0.37 | 1.4[c] ± 0.67 | 2.2[d] ± 0.53 | 1.6 ± 0.55 |

[a]For all serum constituents except A/G.

[b]Abbreviations used in all tables for the following carbohydrates are: G = glucose, F = fructose, S = sucrose, G-S = glucose-sucrose.

[c,d]Means are significantly different from one another ($P < 0.05$).

*Serum Glycoproteins*

While the alpha$_1$ and beta glycoprotein levels did not significantly vary with dietary regimen, the alpha$_2$ glycoprotein levels exhibited a significant variation with Diets 2 and 3 (Table 3). Thus, those animals on the fructose diet (Diet 2) had a higher alpha$_2$ value than those on the sucrose diet (Diet 3). Furthermore, the alpha$_2$ glycoprotein levels were very high when compared with the levels previously reported for rats by Cockerell, *et al.*, (1973) who stated that serum glycoprotein distributions from rats maintained on a regular rat laboratory chow, ranged from 2.7% albumin; 53.4% alpha$_1$ glycoprotein; 15.7% alpha$_2$ glycoprotein; 17.4% beta glycoprotein; and 10.8% gamma glycoproteins. These findings suggest that alterations in serum alpha$_2$ glycoprotein levels may be affected by the amount and type of mono- or disaccharide in the diet.

TABLE 3

Effect of Experimental Diets on Serum Levels of Glycoproteins, Haptoglobin, and Bilirubin.

| Serum Determination | Concentration of Serum Constituents[a] | | | |
|---|---|---|---|---|
| | Diet 1 (G) | Diet 2 (F) | Diet 3 (S) | Diet 4 (G-S) |
| Alpha$_1$ Glycoprotein | 15.20 ± 1.48 | 8.8 ± 8.60 | 17.4 ± 5.59 | 15.2 ± 8.04 |
| Alpha$_2$ Glycoprotein | 74.8 ± 2.86 | 79.0[b] ± 4.53 | 66.8[c] ± 3.27 | 70.6 ± 6.69 |
| Beta Glycoprotein | 10.0 ± 2.82 | 12.2 ± 4.83 | 15.8 ± 5.63 | 14.2 ± 1.92 |
| Haptoglobin | 238.2 ± 115.05 | 463.2 ± 121.46 | 370.8 ± 42.72 | 286.2 ± 76.65 |
| Bilirubin | 0.17 ± 0.14 | 0.30 ± 0.26 | 0.14 ± 0.10 | 0.00 ± 0.00 |

[a]Glycoprotein concentrations are expressed as % of total; Haptoglobin and bilirubin concentrations are expressed as mg %.

[b,c]Means are significantly different from one another ($P < 0.05$).

Additional electrophoretic studies were conducted to elucidate the nature of the high alpha$_2$ glycoproteins. Rather high levels of haptoglobin, a hemoglobin-binding protein which migrates with the alpha$_2$ fraction were observed (Table 3). There appears to be no significant correlation of the haptoglobin level with the type of carbohydrate in the diet, even though there is an apparent trend toward higher haptoglobin in animals fed fructose (Diet 2).

*Serum Bilirubin and Erythrocyte Osmotic Fragility*

The levels of serum bilirubin (Table 3) varied from 0.0 to 0.3 mg%. The latter value, obtained for fructose-fed animals, parallels the results for the haptoglobin studies. However, this determination may not accurately reflect the true bilirubin content due to the large standard deviation. Several investigators (Berk, *et al.*, 1969; Grodsky, *et al.*, 1962; Ostrow, *et al.*, 1962, 1963; With, 1954) have shown that hepatic uptake and biliary excretion of bilirubin are much more rapid in rats than in man due to the relatively greater size of the liver, a higher metabolic rate, and differences in hepatic blood flow. Thus, serum bilirubin levels may not respond to a high dietary intake of mono- or disaccharides.

The response of erythrocytes from rats on the different diets to *in vitro* hypotonic saline solutions is presented in Table 4. Although other investigators have studied rat erythrocyte hemolysis in relation to the ratio of tocopherols to polyunsaturated fatty acids in the diet (Bieri and Evarts, 1975; Bieri and Poukka, 1970), published studies are not available revealing rat erythrocyte osmotic fragility in hypotonic saline solution. Thus, the only comparison that could be made in this investigation was to that of hemolysis in man reported by Davidson, *et al.*, (1969) shown in Table 4. No erythrocyte response was noted for NaCl concentrations ranging from 0.85 to 0.60%. However, upon varying the NaCl concentration from 0.55 to 0.30%, erythrocyte hemolysis occurred in all animals. Of all the groups of animals, those on Diet 2 (fructose) displayed an apparently greater tendency toward erythrocyte hemolysis (Table 4).

TABLE 4

Effect of Experimental Diets on Per Cent Range of Erythrocyte Osmotic Fragility

| Concentration of NaCl | % Erythrocyte Hemolysis | | | | |
|---|---|---|---|---|---|
| | Diet 1 (G) | Diet 2 (F) | Diet 3 (S) | Diet 4 (G-S) | Man[a] |
| 0.55% | 2.2 ± 2.4 | 9.6 ± 5.0 | 6.0 ± 4.5 | 0.3 ± 0.6 | 0 |
| 0.50% | 57.6 ± 11.9 | 52.6 ± 11.8 | 50.4 ± 11.1 | 28.0 ± 14.8 | 0-5 |
| 0.45% | 78.8 ± 12.4 | 90.1 ± 6.8 | 82.0 ± 14.9 | 74.7 ± 5.5 | 5-45 |
| 0.40% | 83.0 ± 9.0 | 97.7 ± 3.1 | 86.2 ± 7.1 | 97.4 ± 3.8 | 50-90 |
| 0.30% | 84.4 ± 10.3 | 98.5 ± 2.8 | 94.8 ± 2.4 | 98.6 ± 3.2 | 97-100 |

[a]Davidson and Henry (1969)


## SUMMARY

The effects of dietary carbohydrates were studied on certain serum constituents (e.g., total protein, albumin, the globulins, glycoprotein distribution, haptoglobin, bilirubin) as well as on erythrocyte osmotic fragility. Rats were maintained for 28 days on isocaloric, isonitrogenous, chemically defined diets which differed only in the type of carbohydrate (Diet 1, glucose; 2, fructose; 3, sucrose; 4, equal amounts of glucose and sucrose).

Analysis of the serum proteins revealed: (a) total protein levels were least for animals on Diet 1 (glucose), (b) the A/G was lowest for animals on Diet 2 (fructose); (c) the A/G was highest for animals on Diet 3 (sucrose); and (d) beta globulin was highest for animals on Diet 2 (fructose).

Serum glycoprotein electrophoresis revealed high alpha$_2$ glycoprotein levels (% of total) with the highest being observed for animals on the fructose diet (Diet 2). The serum haptoglobin and bilirubin levels were

also highest for those animals on the same diet. Lastly, it was found that the erythrocytes of these animals (fructose diet) were the most susceptible to hemolysis in hypotonic saline solution.

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# RAYLEIGH-SCHROEDINGER PERTURBATION CALCULATIONS FOR BERYLLIUM[1]

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ABSTRACT

The ground-state energy for beryllium through 2nd order has been calculated using degenerate Rayleigh-Schroedinger perturbation theory. The perturbation terms were the interelectronic potentials. The wave functions used were antisymmetric products of 1-electron atomic eigenfunctions, and were also eigenfunctions of spin. The eigenvalues and eigenvectors of the 1st-order perturbation matrix were obtained. The true zero-order ground-state eigenfunction was then approximated and the 2nd-order energy was obtained. The approximation was the representation of the ground-state by the state $1s^2 2s^2\ ^1S_0$. The total energy through 2nd-order was $E_0 = -14.65885$ which is 0.06% above the experimental energy.

## INTRODUCTION

In the past few years, many new and powerful methods have been applied to the calculation of energy levels of atoms with small values of z. These investigations can be classified according to the type of approximate wave function used as follows:

1. Single-particle orbital
   a. Hartree-Fock
   b. Hartree-Fock-Slater
2. Correlated wave function
   a. Configuration-interaction
   b. Interelectronic coordinates
3. Perturbation
   a. Rayleigh-Schroedinger (R-S)
   b. Brueckner-Goldstone (many-body).

The single-particle orbital method can be further classified as to whether the wave functions used contain variational parameters which are adjusted so as to obtain the minimum total energy for the system, or the variational principle is used to obtain a set of integrodifferential equations which are solved self-consistently,

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yielding the wave function for the system. Recent many-body calculations have used the Hartree-Fock orbitals as a basis set for their perturbation expansion. As would be expected, the perturbation method yields significantly improved results.

The correlated wave function approach is, like the single-particle orbital method, a variational method and involves the expansion of the wave function in terms of either the interelectronic coordinate, $r_{ij}$, or the spherical harmonics, $Y_{\ell}^{m}(\Theta, \Phi)$, in addition to single particle terms and variational parameters.

Normally, Hartree-Fock calculations will give a total energy for the system which differs from the experimental value by less than a few %. Perturbation calculations and correlated wave function calculations will give results 1 or 2 orders of magnitude better than this, depending on the number of terms used, basis set, etc.

Froese (1967) has used the Hartree-Fock method for calculation of energy levels for 2- and 4-electron systems. Barnett and Shull (1967) and Barnett and Platas (1968) have used the Reduced Density-Matrix theory to obtain the ground-state energy of beryllium. Kelly (1963) used the many-body perturbation theory to obtain the ground state of beryllium to within 0.03% of the experimental energy. Weiss (1961) used the configuration-interaction approach to obtain results for the ground state of beryllium which were within 0.05% of the experimental value. Tuan and Sinanoglu (1964) used a many-electron theory to obtain a value for the ground-state energy of beryllium which is in excellent agreement with the experimental value. The above successes together with the supposed difficulty of the Rayleigh-Schroedinger perturbation method have probably caused the neglect of perhaps the oldest of the approximate methods. It is the purpose of this paper to show that the R-S method is indeed viable and can give surprisingly good results, even when applied only through 2nd order.

## DEVELOPMENT OF THE PERTURBATION SERIES

The atomic system chosen is the Be atom, and the level chosen is the ground state. The Hamiltonian in atomic units is

$$H = H_0 + V, \tag{1}$$

where

$$H_0 = -\frac{1}{2}\sum_{i=1}^{4} \nabla_i^2 - \sum_{i=1}^{4} \frac{z}{r_i}, \tag{2}$$

and

$$V = \sum_{\substack{i,j=1 \\ i<j}}^{4} \frac{1}{r_{ij}}. \tag{3}$$

It is required that the eigenfunctions of $H_0$ be antisymmetric and also be eigenfunctions of spin. The ground-state of beryllium (spectroscopic designation) is $1s^2 2s^2\ {}^1S_0$, indicating a spin of zero and a spin projection of zero. The 4-particle spin eigenfunctions which satisfy zero spin and zero spin projection are

$$ {}^1\chi_0^1 = \frac{1}{2}\ [\alpha_1\beta_2\alpha_3\beta_4 - \alpha_1\beta_2\beta_3\alpha_4 - \beta_1\alpha_2\alpha_3\beta_4 - \beta_1\alpha_2\beta_3\alpha_4]\,, \tag{4} $$

and

$$ {}^1\chi_0^2 = \frac{1}{2\sqrt{3}}\,[2\alpha_1\alpha_2\beta_3\beta_4 - \alpha_1\beta_2\alpha_3\beta_4 - \alpha_1\beta_2\beta_3\alpha_4 - \beta_1\alpha_2\alpha_3\beta_4 - \beta_1\alpha_2\beta_3\alpha_4 + 2\beta_1\beta_2\alpha_3\alpha_4]\,. \tag{5} $$

From these spin functions, it is readily deduced that possible eigenfunctions of $H_0$ which satisfy both the Pauli Exclusion principle and the spin requirements are

$$ \Phi_{1i} = N_{1i}\left[\begin{pmatrix} abcd \\ \alpha\beta\alpha\beta \end{pmatrix} - \begin{pmatrix} abcd \\ \alpha\beta\beta\alpha \end{pmatrix} - \begin{pmatrix} abcd \\ \beta\alpha\alpha\beta \end{pmatrix} + \begin{pmatrix} abcd \\ \beta\alpha\beta\alpha \end{pmatrix}\right] \tag{6} $$

and

$$ \Phi_{2i} = N_{2i}\left[2\begin{pmatrix} abcd \\ \alpha\alpha\beta\beta \end{pmatrix} - \begin{pmatrix} abcd \\ \alpha\beta\alpha\beta \end{pmatrix} - \begin{pmatrix} abcd \\ \alpha\beta\beta\alpha \end{pmatrix} - \begin{pmatrix} abcd \\ \beta\alpha\alpha\beta \end{pmatrix} - \begin{pmatrix} abcd \\ \beta\alpha\beta\alpha \end{pmatrix} + 2\begin{pmatrix} abcd \\ \beta\beta\alpha\alpha \end{pmatrix}\right], \tag{7} $$

where the a, b, c, and d represent 1 electron atomic eigenfunctions and

$$ \begin{pmatrix} abcd \\ \alpha\beta\alpha\beta \end{pmatrix} = \frac{1}{\sqrt{24}}\begin{vmatrix} a_1\alpha_1 & a_2\alpha_2 & a_3\alpha_3 & a_4\alpha_4 \\ b_1\beta_1 & b_2\beta_2 & b_3\beta_3 & b_4\beta_4 \\ c_1\alpha_1 & c_2\alpha_2 & c_3\alpha_3 & c_4\alpha_4 \\ d_1\beta_1 & d_2\beta_2 & d_3\beta_3 & d_4\beta_4 \end{vmatrix} \tag{8} $$

The $N_{1i}$ and $N_{2i}$ are the normalization coefficients. By expanding the determinants, one can show that

$$ \Phi_{1i} = \frac{N_{1i}}{\sqrt{24}}\left[{}^1\chi_0^1\ (2A - B - C) + \sqrt{3}\ {}^1\chi_0^2\,(C - B)\right], \tag{9} $$

and

$$ \Phi_{2i} = \frac{N_{1i}}{\sqrt{24}}\left[{}^1\chi_0^2\ (2\sqrt{3}\ G - \sqrt{3}\ H - \sqrt{3}\ I) + {}^1\chi_0^1\,(3H - 3I)\right], \tag{10} $$

where

$$A = abcd + bacd + abdc + badc + cdab + dcab + dcba + cdba,$$

$$B = acbd + bcad + adbc + bdac + cadb + cbda + dacb + dbca,$$

$$C = acdb + bcda + adcb + bdca + cabd + cbad + dabc + dcab,$$

$$G = abcd + cdab - abdc - cdba - bacd - dcab + badc + dcba,$$

$$H = adbc + cbda - acbd - cadb + bcad + dacb - bdac - dbca,$$

and

$$I = acdb + cabd - adcb - cbad - bcda - dabc + bdca + dbac.$$

The subscripts indicating the particle coordinates have now been dropped and the coordinates will be designated by position. Thus

$$abcd = a_1 b_2 c_3 d_4 .$$

Eigenfunctions have now been found which are both antisymmetric and eigenfunctions of spin. In particular the eigenfunctions have a spin of zero and a spin projection of zero.

It is easily shown that $\Phi_{1i}$ and $\Phi_{2i}$ are orthogonal only when none of the a, b, c, d, is equal to another of the a, b, c, d. Whenever any 2 of the a, b, c, d are equal $\Phi_{1i} = \Phi_{2i}$, but if 3 or 4 of the a, b, c, d are equal then $\Phi_{1i} = \Phi_{2i} = 0$. The only other possibility is for the a, b, c, d to form 2 equal pairs in which case $\Phi_{2i} = 0$ but $\Phi_{1i} \neq 0$.

The ground state eigenfunction is obtained by the choice $a = U_{100}$, $b = U_{100}$, $c = U_{200}$ and $d = U_{200}$. Then Equations (6) and (7) become

$$\Phi_{10} = 4N_{10} \begin{pmatrix} U_{100} & U_{100} & U_{200} & U_{200} \\ \alpha & \beta & \alpha & \beta \end{pmatrix} = \Phi_{\sigma}, \tag{11}$$

and

$$\Phi_{20} = 0. \tag{12}$$

The normalization coefficients are easily calculated and are

$$N_{1i} = 1/(4 - 2\Delta_{ac} - 2\Delta_{bc} - 2\Delta_{bd} + 4\Delta_{ab} + 4\Delta_{cd} + 4\Delta_{ab}\Delta_{cd} + 4\Delta_{ac}\Delta_{bd} + 4\Delta_{ad}\Delta_{bc})^{1/2}, \tag{13a}$$

and

$$N_{2i} = 1/[6(2 - 2\Delta_{ab} - 2\Delta_{cd} + \Delta_{ac} + \Delta_{bd} + \Delta_{bc} + \Delta_{ad} + 2\Delta_{ab}\Delta_{cd} + 2\Delta_{ad}\Delta_{bc} + 2\Delta_{ac}\Delta_{bd})]^{1/2}, \tag{13b}$$

where $\Delta_{ab} = \delta_{nn'}\delta_{\ell\ell'}\delta_{mm'}$ whenever $a = U_{n\ell m}$ and $b = U_{n'\ell'm'}$.

The zero order ground state energy will now be found. Thus

$$H_O|\Phi_O> = E_O^{(0)}|\Phi_O> \tag{14}$$

or

$$E_O^{(0)} = \frac{<\Phi_O|H_O|\Phi_O>}{<\Phi_O|\Phi_O>} \tag{15}$$

$$= \frac{1}{24}\,[<2D-E-F\,|H_O|\,2D-E-F> + 3<F-E\,|H_O|\,F-E>\,], \tag{16}$$

where

$$D = U_{100}^{(1)}\ U_{100}^{(2)}\ U_{200}^{(3)}\ U_{200}^{(4)} + U_{200}^{(1)}\ U_{200}^{(2)}\ U_{100}^{(3)}\ U_{100}^{(4)},$$

$$E = U_{100}^{(1)}\ U_{200}^{(2)}\ U_{100}^{(3)}\ U_{200}^{(4)} + U_{200}^{(1)}\ U_{100}^{(2)}\ U_{200}^{(3)}\ U_{100}^{(4)},$$

and

$$F = U_{100}^{(1)}\ U_{200}^{(2)}\ U_{200}^{(3)}\ U_{100}^{(4)} + U_{200}^{(1)}\ U_{100}^{(2)}\ U_{100}^{(3)}\ U_{200}^{(4)}.$$

Equation (16) is easily evaluated and gives

$$E_O^{(0)} = \left(-\frac{z^2}{2}-\frac{z^2}{2}-\frac{z^2}{8}-\frac{z^2}{8}\right)\ \left(<2D-E-F\,|2D-E-F> + 3<F-E\,|F-E>\right)/24$$

$$= -\frac{5z^2}{4},$$

and for $z = 4$,

$$E_O^{(0)} = -20.$$

It is found that the 10 eigenfunctions of $H_O$ listed in Table 1 also correspond to this energy. The ground state is thus degenerate, and degenerate perturbation theory must be used. The first order correction (Kelly, 1968) to the energy and the expansion coefficients for the proper wave function are obtained by determining the eigenvalues and corresponding eigenvectors of the perturbation matrix

$$V_{n_i n_j} - E_n^{(1)}\,\delta_{n_i n_j} = 0, \tag{17}$$

where

$$V_{n_i n_j} = <\Phi_{n_i}|V|\Phi_{n_j}>. \tag{18}$$

TABLE 1

Ground State Eigenfunctions.

| a | b | c | d | $\Phi$ |
|---|---|---|---|---|
| $U_{100}$ | $U_{100}$ | $U_{200}$ | $U_{200}$ | $\Phi_{01}$ |
| $U_{100}$ | $U_{100}$ | $U_{200}$ | $U_{21^{-1}}$ | $\Phi_{02}$ |
| $U_{100}$ | $U_{100}$ | $U_{200}$ | $U_{210}$ | $\Phi_{03}$ |
| $U_{100}$ | $U_{100}$ | $U_{200}$ | $U_{211}$ | $\Phi_{04}$ |
| $U_{100}$ | $U_{100}$ | $U_{21^{-1}}$ | $U_{21^{-1}}$ | $\Phi_{05}$ |
| $U_{100}$ | $U_{100}$ | $U_{21^{-1}}$ | $U_{210}$ | $\Phi_{06}$ |
| $U_{100}$ | $U_{100}$ | $U_{21^{-1}}$ | $U_{211}$ | $\Phi_{07}$ |
| $U_{100}$ | $U_{100}$ | $U_{210}$ | $U_{210}$ | $\Phi_{08}$ |
| $U_{100}$ | $U_{100}$ | $U_{210}$ | $U_{211}$ | $\Phi_{09}$ |
| $U_{100}$ | $U_{100}$ | $U_{211}$ | $U_{211}$ | $\Phi_{10}$ |

Using the notation of Table 1 and for i = j = 1 Equation 18 becomes

$$V_{11} = \langle \Phi_{01} | V | \Phi_{01} \rangle$$

$$= \frac{1}{24} \left( \langle 2D - E - F | V | 2D - E - F \rangle + 3 \langle F - E | V | F - E \rangle \right)$$

$$= \frac{1}{6} \left( \langle D | V | D \rangle + \langle E | V | E \rangle + \langle F | V | F \rangle - \langle D | V | E \rangle - \langle D | V | F \rangle \right.$$

$$\left. - \langle E | V | F \rangle \right) . \tag{19}$$

After expansion and integration, Equation (19) becomes

$$V_{11} = \frac{5z}{8} + \frac{77z}{512} + \frac{68z}{81} - \frac{32z}{729} , \tag{20}$$

and for z = 4

$$V_{11} = 6.28400420 \tag{21}$$

Equation (18) is given explicitly in Table 2.

The diagonalization is easily performed and the results are given in Table 3.

Using the results of Table 3 the proper ground state eigenfunction of $H_o$ can now be written. It has the form

$$\Phi_0^1 = \sum_{i=1}^{10} C_i \Phi_{oi},$$

where the $C_i$'s make up the eignevector corresponding to the lowest eigenvalue

TABLE 2

The $V_{n_i n_j}$ Matrix.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 6.284004 | 0 | 0 | 0 | 0 | 0 | -0.165728 | 0.117187 | 0 | 0 |
| 0 | 6.730950 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 6.730950 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 6.730950 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 6.988834 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 6.904459 | 0 | 0 | 0 | 0 |
| -0.165728 | 0 | 0 | 0 | 0 | 0 | 7.073209 | -0.059662 | 0 | 0 |
| -0.117187 | 0 | 0 | 0 | 0 | 0 | -0.059662 | 7.031021 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.904459 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.988834 |

TABLE 3

Eigenvalues and Eigenvectors.

| $E_0^{(1)}$ | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ | $C_7$ | $C_8$ | $C_9$ | $C_{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 6.237097 | 0.974321 | 0 | 0 | 0 | 0 | 0 | 0.183846 | -0.129999 | 0 | 0 |
| 6.730950 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.730950 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.730950 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.988834 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 6.904459 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 7.162304 | -0.225165 | 0 | 0 | 0 | 0 | 0 | 0.795529 | -0.562524 | 0 | 0 |
| 6.988834 | 0 | 0 | 0 | 0 | 0 | 0 | 0.577350 | 0.816497 | 0 | 0 |
| 6.904459 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 6.988834 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

of Equation (17). Explicitly

$$\Phi_0^1 = 0.9743201\,\Phi_{01} + 0.1838465\,\Phi_{07} + 0.1299991\,\Phi_{08}\,.$$

The 2nd order ground-state energy may now be obtained from the well-known equation

$$E_O^{(2)} = \Sigma'_{i,m} \frac{|\langle\Phi_0^1|V|\Phi_{im}\rangle|^2}{E_O^{(0)} - E_m^{(0)}}, \tag{22}$$

where i = 1 or i = 2 and the prime on the $\Sigma$ indicates that the sum over the $\Phi_{im}$ does not include any of the $\Phi_{oi}$'s. Since $\langle\Phi_{01}|V|\Phi_{im}\rangle$, $\langle\Phi_{07}|V|\Phi_{im}\rangle$ and $\langle\Phi_{08}|V|\Phi_{im}\rangle$ are all close to the same value, and since approximately 95% of the energy contribution will come from $\langle\Phi_{01}|V|\Phi_{im}\rangle$, the approximation is made that $C_1 = 1$, $C_7 = C_8 = 0$. Then Equation (22) becomes

$$E_O^{(2)} = \Sigma' \frac{|\langle\Phi_{01}|V|\Phi_{1m}\rangle|^2}{E_O^{(0)} - E_m^{(0)}} + \Sigma' \frac{|\langle\Phi_{01}|V|\Phi_{2m}\rangle|^2}{E_O^{(0)} - E_m^{(0)}}, \tag{23}$$

where the $\Sigma$ also includes integration over continuum states.

It is possible to calculate $E_m^{(0)}$. The result is

$$E_m^{(0)} = -\frac{z^2}{2}\left[\frac{1}{n_1^2} + \frac{1}{n_2^2} + \frac{1}{n_3^2} + \frac{1}{n_4^2}\right], \tag{24}$$

where $n_1$, $n_2$, $n_3$, and $n_4$ are the principal quantum numbers of the 1-electron atomic eigenfunctions which make up the $\Phi_{im}$.

The matrix elements of Equation (23) may now be expanded and rewritten using symmetry arguments. The results are

$$\langle\Phi_{01}|V|\Phi_{1m}\rangle = \frac{N_{1m}}{4}\left[\langle D|\frac{1}{r_{12}}|4A-2B-2C\rangle + \langle E|\frac{1}{r_{12}}|4B-2A-2C\rangle + \langle F|\frac{1}{r_{12}}|4C-2B-2A\rangle\right], \tag{25a}$$

and

$$\langle\Phi_{01}|V|\Phi_{2m}\rangle = \frac{3N_{2m}}{2}\left[\langle D|\frac{1}{r_{12}}|H-I\rangle + \langle E|\frac{1}{r_{12}}|I-G\rangle + \langle F|\frac{1}{r_{12}}|G-H\rangle\right]. \tag{25b}$$

The notation $U_{100} = 1$, $U_{200} = 2$, $U_{210} = 3$, $U_{21-1} = 4$, and $U_{211} = 5$ is helpful. Then

$$\langle U^{(1)}_{100}\, U^{(2)}_{100}\, U^{(3)}_{200}\, U^{(4)}_{200} \,|abcd\rangle = \langle 1122\,|abcd\rangle = \Delta_{1a}\,\Delta_{1b}\,\Delta_{2c}\,\Delta_{2d}$$

Since the terms of the perturbation are 2-particle interactions but the eigenfunctions of $H_o$ are 4-particle eigenfunctions, the matrix elements of Equation (23) will vanish unless 2 of the a, b, c, and d are either $U_{100}$ or $U_{200}$. The Equations (25a) and (25b) then become

$$\begin{aligned}\langle\Phi_{01}\,|V|\,\Phi_{1m}\rangle \;=\; \frac{N_1}{4}\;\Big(&\Delta_{1a}\Big[16\langle 22\,|v|\,cd\rangle\Delta_{1b} - 8\langle 22\,|v|\,bc\rangle\Delta_{1d}\\
&-8\langle 22|v|bd\rangle\Delta_{1c} + \Delta_{2d}(16\langle 12|v|bc\rangle - 8\langle 12|v|cb\rangle)\\
&+\Delta_{2c}(16\langle 12|v|bd\rangle - 8\langle 12|v|db\rangle) + \Delta_{2b}(-8\langle 12|v|cd\rangle\\
&-8\langle 12|v|dc\rangle)\Big] + \Delta_{2a}\Big[16\langle 11|v|cd\rangle\Delta_{2b} - 8\langle 11|v|bc\rangle\Delta_{2d}\\
&-8\langle 11|v|bd\rangle\Delta_{2c} + \Delta_{1d}(16\langle 12|v|cb\rangle - 8\langle 12|v|bc\rangle)\\
&+\Delta_{1c}(16\langle 12|v|db\rangle - 8\langle 12|v|bd\rangle) + \Delta_{1b}(-8\langle 12\,|v|dc\rangle\\
&-8\langle 12|v|cd\rangle)\Big] + \Delta_{1c}\Big[16\langle 22\,|v|\,ab\rangle\Delta_{1d} - 8\langle 22|v|ad\rangle\Delta_{1b}\\
&+\Delta_{2b}(16\langle 12|v|da\rangle - 8\langle 12|v|ad\rangle) + \Delta_{2d}(-8\langle 12|v|ab\rangle\\
&-8\langle 12|v|ba\rangle)\Big] + \Delta_{2c}\Big[16\langle 11|v|ab\rangle\Delta_{2d} - 8\langle 11|v|ad\rangle\Delta_{2b}\\
&+\Delta_{1b}(16\langle 12|v|ad\rangle - 8\langle 12|v|da\rangle) + \Delta_{1d}(-8\langle 12|v|ab\rangle\\
&-8\langle 12|v|ba\rangle)\Big] + \Delta_{1b}\Big[-8\langle 22|v|ac\rangle\Delta_{1d} + \Delta_{2d}(16\langle 12|v|ac\rangle\\
&-8\langle 12|v|ca\rangle)\Big] + \Delta_{2b}\Big[-8\langle 11|v|ac\rangle\Delta_{2d} + \Delta_{1d}(16\langle 12|v|ac\rangle\\
&-8\langle 12|v|ca\rangle)\Big]\Big)\,, \qquad (26a)\end{aligned}$$

and

$$\begin{aligned}\langle\Phi_{01}\,|V|\,\Phi_{2m}\rangle = \frac{3N_2}{2}\Big(&\Delta_{1a}\Big[\Delta_{2b}(4\langle 12|v|dc\rangle - 4\langle 12|v|cd\rangle) + 4\langle 12|v|db\rangle\Delta_{2c}\\
&-4\langle 12|v|cb\rangle\Delta_{2b} + 4\langle 22|v|cb\rangle\Delta_{1d} - 4\langle 22|v|db\rangle\Delta_{1c}\Big]\\
&+\Delta_{2a}\Big[\Delta_{1b}(4\langle 12|v|cd\rangle - 4\langle 12|v|dc\rangle) + 4\langle 12|v|bd\rangle\Delta_{1c}\end{aligned}$$

$$-4\langle 12|v|bc\rangle\Delta_{1d}+4\langle 11|v|bc\rangle\Delta_{2d}-4\langle 11|v|bd\rangle\Delta_{2c}\Big]$$
$$+\Delta_{1b}\Big[4\langle 12|v|ca\rangle\Delta_{2d}-4\langle 12|v|da\rangle\Delta_{2c}+4\langle 22|v|ad\rangle\Delta_{1c}$$
$$-4\langle 22|v|ac\rangle\Delta_{1d}\Big]+\Delta_{2b}\Big[4\langle 12|v|ac\rangle\Delta_{1d}-4\langle 12|v|ad\rangle\Delta_{1c}$$
$$+\langle 11|v|ad\rangle\Delta_{2c}-4\langle 11|v|ac\rangle\Delta_{2d}\Big]+\Delta_{1c}\Delta_{2d}(4\langle 12|v|ba\rangle$$
$$-4\langle 12|v|ab\rangle)+\Delta_{2c}\Delta_{1d}(4\langle 12|v|ab\rangle-4\langle 12|v|ba\rangle)\Big]\Big) \quad (26b)$$

For the case of a = 1, c = 2, and d ≠ 1, 2, Equations (23), (24) and (26a) may be combined to give

$$E_{01}^{(2)} = \Sigma' \frac{2|\langle 22|v|2d\rangle + 2\langle 12|v|1d\rangle - \langle 12|v|d1\rangle|^2}{\frac{-z^2}{8} + \frac{z^2}{2n^2}} \quad (27)$$

where $v = \frac{1}{r_{12}}$. Equation (27) may be written more explicitly. In particular

$$E_{01}^{(2)} = \sum_{\substack{n\ell m \\ n>2}} 2\Big[\langle U_{200}^{(1)}U_{200}^{(2)}|\frac{1}{r_{12}}|U_{200}^{(1)}U_{n\ell m}^{(2)}\rangle + 2\langle U_{100}^{(1)}U_{200}^{(2)}|\frac{1}{r_{12}}|U_{100}^{(1)}U_{n\ell m}^{(2)}\rangle$$
$$- \langle U_{100}^{(1)}U_{200}^{(2)}|\frac{1}{r_{12}}|U_{n\ell m}^{(1)}U_{100}^{(2)}\rangle\Big]^2 \Big/ \Big[-\frac{z^2}{8} + \frac{z^2}{2n^2}\Big]$$
$$+\int_0^\infty dk\, 2\Big[\langle U_{200}^{(1)}U_{200}^{(2)}|\frac{1}{r_{12}}|U_{200}^{(1)}U_{k\ell m}^{(2)}\rangle + 2\langle U_{100}^{(1)}U_{200}^{(2)}|\frac{1}{r_{12}}|U_{100}^{(1)}U_{k\ell m}^{(2)}\rangle$$
$$- \langle U_{100}^{(1)}U_{200}^{(2)}|\frac{1}{r_{12}}|U_{k\ell m}^{(1)}U_{100}^{(2)}\rangle\Big]^2 \Big/ \Big[-\frac{z^2}{8} - \frac{k^2}{2}\Big] . \quad (28)$$

There are 16 other cases similar to Equation (28). These are listed in Table 4, and

$$E_0^{(2)} = \sum_{i=1}^{17} E_{oi}^{(2)} \quad (29)$$

These series have been evaluated for $\ell \leq 4$ and the results tabulated in Table 5.

The ground-state energy has now been obtained and the result is

$$E_0 = E_0^{(0)} + E_0^{(1)} + E_0^{(2)} + \ldots\ldots$$
$$= -20 + 6.237097 - 0.83595$$
$$= -14.65885 \quad (30)$$

or in terms of z

$$E_0 = \frac{5}{4}z^2 + 1.559274z - 0.83595.$$

TABLE 4

The Energy Series.

| Case | Series |
|---|---|
| 112n | $E_{01}^{(2)} = \Sigma' \dfrac{2\left\lvert\langle 22\rvert v\rvert 2d\rangle + 2\langle 12\rvert v\rvert 1d\rangle - \langle 12\rvert v\rvert d1\rangle\right\rvert^2}{-\frac{z^2}{8} + \frac{z^2}{2n^2}}$ |
| 11pn | $E_{02}^{(2)} = \Sigma' \dfrac{2\left\lvert\langle 22\rvert v\rvert pn\rangle\right\rvert^2}{-\frac{z^2}{8} + \frac{z^2}{2n^2}}$ $p = 3, 4, 5$ |
| $11nn'$ | $E_{03}^{(2)} = \sum'_{n \geq n'} \dfrac{1}{1+\Delta_{cd}} \dfrac{2\left\lvert\langle 22\rvert v\rvert cd\rangle\right\rvert^2}{-\frac{z^2}{4} + \frac{z^2}{2}\left(\frac{1}{n_1^2} + \frac{1}{n_2^2}\right)}$ |
| 22pn | $E_{04}^{(2)} = \Sigma' \dfrac{2\left\lvert\langle 11\rvert v\rvert pn\rangle\right\rvert^2}{-\frac{7z^2}{8} + \frac{z^2}{2n^2}}$ $p = 3, 4, 5$ |
| $22nn'$ | $E_{05}^{(2)} = \sum'_{n \geq n'} \dfrac{1}{1+\Delta_{cd}} \dfrac{2\left\lvert\langle 11\rvert v\rvert cd\rangle\right\rvert^2}{-z^2 + \frac{z^2}{2}\left(\frac{1}{n_1^2} + \frac{1}{n_2^2}\right)}$ |
| 223p | $E_{06}^{(2)} = \dfrac{1}{1+\Delta_{3p}} \dfrac{2\left\lvert\langle 11\rvert v\rvert 3p\rangle\right\rvert^2}{-\frac{3z^2}{4}}$ $p = 3, 4, 5$ |
| 224p | $E_{07}^{(2)} = \dfrac{1}{1+\Delta_{4p}} \dfrac{2\left\lvert\langle 11\rvert v\rvert 4p\rangle\right\rvert^2}{-\frac{3z^2}{4}}$ $p = 4, 5$ |
| 2255 | $E_{08}^{(2)} = \dfrac{\left\lvert\langle 11\rvert v\rvert 55\rangle\right\rvert^2}{-\frac{3z^2}{4}}$ |
| 122n | $E_{09}^{(2)} = \Sigma' \dfrac{2\left\lvert\langle 11\rvert v\rvert 1d\rangle + 2\langle 12\rvert v\rvert d2\rangle - \langle 12\rvert v\rvert 2d\rangle\right\rvert^2}{-\frac{z^2}{2} + \frac{z^2}{2n^2}}$ |
| 12pn | $E_{010_a}^{(2)} = \dfrac{\left\lvert\langle 12\rvert v\rvert pn\rangle + \langle 12\rvert v\rvert np\rangle\right\rvert^2}{-\frac{z^2}{2} + \frac{z^2}{2n^2}}$ $p = 3, 4, 5$ |
| | $E_{010_b}^{(2)} = \dfrac{3\left\lvert\langle 12\rvert v\rvert np\rangle - \langle 12\rvert v\rvert pn\rangle\right\rvert^2}{-\frac{z^2}{2} + \frac{z^2}{2n^2}}$ |
| 122p | $E_{011}^{(2)} = \dfrac{2\left\lvert\langle 12\rvert v\rvert 2p\rangle - 2\langle 12\rvert v\rvert p2\rangle - \langle 11\rvert v\rvert 1p\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ $p = 3, 4, 5$ |

TABLE 4 (Continued)

| Case | Series |
|---|---|
| 1233 | $E^{(2)}_{012} = \dfrac{2\left\lvert\langle 12\rvert v\rvert 33\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ |
| 123p | $E^{(2)}_{013_a} = \dfrac{\left\lvert\langle 12\rvert v\rvert 3p\rangle + \langle 12\rvert v\rvert p3\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ $p = 4, 5$ |
| | $E^{(2)}_{013_b} = \dfrac{3\left\lvert\langle 12\rvert v\rvert p3\rangle - \langle 12\rvert v\rvert 3p\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ $p = 4, 5$ |
| 1244 | $E^{(2)}_{014} = \dfrac{2\left\lvert\langle 12\rvert v\rvert 44\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ |
| 1245 | $E^{(2)}_{015_a} = \dfrac{\left\lvert\langle 12\rvert v\rvert 45\rangle + \langle 12\rvert v\rvert 54\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ |
| | $E^{(2)}_{015_b} = \dfrac{3\left\lvert\langle 12\rvert v\rvert 45\rangle - \langle 12\rvert v\rvert 54\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ |
| 1255 | $E^{(2)}_{016} = \dfrac{2\left\lvert\langle 12\rvert v\rvert 55\rangle\right\rvert^2}{-\frac{3z^2}{8}}$ |
| 12nn′ | $E^{(2)}_{017_a} = \Sigma' \dfrac{1}{1+\Delta_{cd}} \dfrac{\left\lvert\langle 12\rvert v\rvert cd\rangle + \langle 12\rvert v\rvert dc\rangle\right\rvert^2}{-\frac{5z^2}{8} + \frac{z^2}{2}\left(\frac{1}{n_1^2} + \frac{1}{n_2^2}\right)}$ |
| | $E^{(2)}_{017_b} = \sum_{c\neq d} \dfrac{3}{1-\Delta_{cd}} \dfrac{\left\lvert\langle 12\rvert v\rvert dc\rangle - \langle 12\rvert v\rvert cd\rangle\right\rvert^2}{-\frac{5z^2}{8} + \frac{z^2}{2}\left(\frac{1}{n_1^2} + \frac{1}{n_2^2}\right)}$ |

[a]This series comes from the 4-determinant excited state wave function.

[b]This series comes from the 6-determinant excited state wave function.

## RESULTS AND CONCLUSIONS

The result obtained in Equation (30) is within 0.06% of the experimental value, and is compared with other methods of calculation in Table 6. While the above results are not as close to the experimental value as some of those listed in Table 6, the result was quite close to the experimental value, and the method has several

TABLE 5

Energy Contributions to $E_0^{(2)}$.

| Case | BB | BC | CC | Extrapolation | Total |
|---|---|---|---|---|---|
| $E_{01}$ | -0.50535 | -0.211732 | 0 | -0.01231 | -0.72940 |
| $E_{02}$ | -0.00034 | -0.006749 | 0 | -0.000120 | -0.007209 |
| $E_{03}$ | -0.0071829 | -0.003136 | -0.031177 | -0.000803 | -0.042299 |
| $E_{04}$ | -0.001047 | -0.004977 | 0 | 0 | -0.006024 |
| $E_{05}$ | -0.0004343 | -0.004221 | -0.004064 | -0.000293 | -0.009012 |
| $E_{06}$ | -0.003497 | 0 | 0 | 0 | -0.003497 |
| $E_{07}$ | -0.006994 | 0 | 0 | 0 | -0.006994 |
| $E_{08}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{09}$ | -0.018124 | -0.056885 | 0 | -0.00927 | -0.075936 |
| $E_{010_a}$ | -0.000541 | -0.000363 | 0 | -0.000017 | -0.000921 |
| $E_{010_b}$ | -0.000744 | -0.002363 | 0 | -0.000052 | -0.003159 |
| $E_{011}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{012}$ | -0.000884 | 0 | 0 | 0 | -0.000884 |
| $E_{013_a}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{013_b}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{014}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{015_a}$ | -0.001767 | 0 | 0 | 0 | -0.001767 |
| $E_{015_b}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{016}$ | 0 | 0 | 0 | 0 | 0 |
| $E_{017_a}$ | -0.000132 | -0.000927 | -0.006075 | -0.000055 | -0.007189 |
| $E_{017_b}$ | -0.000004 | -0.000645 | -0.000989 | -0.000026 | -0.001664 |
| Total | | | | | -0.89595 |

[a]This series comes from the 4-determinant excited state wave function.

[b]This series comes from the 6-determinant excited state wave function.

TABLE 6

Comparison of Results for Beryllium.

| Author (Reference) | Result | Method |
|---|---|---|
| Froese (1967) | -14.57307 | Hartree-Fock |
| Weiss (1961) | -14.66090 | Configuration-Interaction |
| Kelly (1963) | -14.6640 | Many-body Perturbation Theory |
| Sinanoglu (1964) | -14.66737 | Many-electron Theory |
| Barnett (1967) | -14.6861<br>-14.6609 | Reduced Density Matrix |
| Barr (1969) | -14.659 | Consolidated Configuration-Interaction Perturbation |
| Saxon (Present Work) | -14.65885 | Rayleigh-Schroedinger Perturbation Theory (Through Second-Order) |
| Experimental | -14.667306 | |

inherent advantages. There is nothing sophisticated about the method itself in that the theory is straightforward and the results are not dependent upon a fortuitous choice of beginning eigenfunctions. Large computing machines are not necessary for the calculations. Preliminary calculations for the above were performed on a Honeywell 1200 with a 4000 word memory and the final calculations were performed on a Honeywell 1250 wihich has a 10,000 word memory. For the 2nd-order energy, wavefunctions of any z may be used, thus eliminating much duplication of effort for atoms with different z.

The R-S method is seen to provide a relatively simple way of obtaining the ground-state energies of 4-electron systems. Excited states should follow similarly, and some of the matrix elements obtained in the calculation of the ground-state will be needed in some of the excited states. The above method can easily be applied to lithium and other few-electron systems.

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# SYNTHESIS OF A RADIOLABELED CARRIER TO PRECIPITATE ANTI-HAPTEN ANTIBODIES

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ABSTRACT

The synthesis of a simple hapten carrier (1,3-dihydroxy-5-iodobenzene) was developed for routine use in the precipitation of anti-hapten antibodies. Coupling to a model hapten (lactose) and quantitative precipitation studies were performed, to apply the compound to the lactoside chicken anti-lactoside antiserum system.

## INTRODUCTION

Pauling, *et al.,* (1942) have shown that some antibodies can be precipitated by small carrier molecules covalently bonded to at least 3 haptenic groups. These synthetic precipitating antigens are chemically defined by their synthesis steps, while natural antigens, such as polypeptides or polysaccharides, have haptenic groups whose 3-dimensional structure is more difficult to identify; often, several different haptenic groups may be present on the same molecule. Our studies of inhibition of precipitation of antigen-antibody complexes required a chemically defined precipitating antigen (to relate antibody avidity to hapten structural formula) which could be precisely assayed in very small quantities (to minimize the amount of serum used). The first problem we wanted to investigate was the influence of the aglycone portion of lactosides on the binding by anti-lactoside antibodies. The chicken had been chosen over the rabbit or the guinea pig to produce the anti-lactoside antibodies because chicken anti-lactoside antisera with consistently high titers were obtained at lower cost.

The most convenient way to covalently bind 3 lactoside haptens to a carrier was through *p*-diazophenyl bridges, since *p*-aminophenyl lactoside could be prepared (Karush, 1957). An activated aromatic carrier such as a phenol analog was desirable to increase the yield of tri-substituted product.

Phloroglucinol has been used as a carrier for glucoside, galactoside, and lactoside haptens (Yariv, *et al.,* 1962), but radiolabeled phloroglucinol is not available. Tritiated phloroglucinol could not be used, since the 3 ring hydrogens are substituted by the haptens, and the 3 phenolic hydrogens would be lost through ionization at the pH most favorable for coupling. Methyl-$^{14}$C methoxy analogs of

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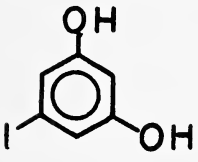
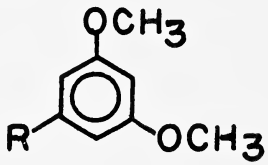
phloroglucinol are less soluble and less reactive than phloroglucinol, so we selected 1,3-dihydroxy-5-iodobenzene as the carrier. Although less soluble and less reactive than phloroglucinol, it could be synthesized with relative ease, and the short half-life of $^{131}I$ and $^{125}I$ minimized decontamination procedures. Of several possibilities which we tried, the simplest overall pathway of synthesis for the model compound, 2,4,6-tri-(*p*-diazophenyl lactoside)-1,3-dihydroxy-5-iodobenzene-$^{125}I$, is outlined below.




1

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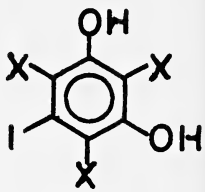


3

3,5-Dimethoxyaniline (2, R = $NH_2$; 1.53 g, 10 mmoles) was diazotized and treated with 1.66 g (10 mmoles) of KI (with or without labeled iodide) to give 0.9 g of 1,3-dimethoxy-5-iodobenzene (2, R = I), mp 70-3 C, after steam distillation and recrystallization from ether. A 0.1 g sample of 1,3-dimethoxy-5-iodobenzene was stirred for 1 hr at 120 C with 1 ml of glacial acetic acid and 1 ml of 57% hydriodic acid, freshly distilled in the presence of hypophosphorous acid. The excess acetic and hydriodic acids were removed under reduced pressure and the residue was dissolved in ether; the ether solution was washed with 50% (w/v) aqueous hypophosphorous acid, diluted $NaHCO_3$, and water, and dried over anhydrous $CaCl_2$. Distillation of the ether at atmospheric pressure, followed by vacuum distillation (0.5 torr) at 120 C, recrystallization from aqueous ethanol and purification by thin layer chromatography (Rf = 0.88; silica gel G; benzene: acetic acid = 5:1, v/v; Rf of compound 2 (R = I) was 0.99) yielded 0.02 g of 1,3-dihydroxy-5-iodobenzene (1), mp 110 C. The reported melting point of 1 is 95 C. (Hodgson and Wignall, 1926); the product we prepared had the proper elemental analysis and the expected NMR and IR spectra for 1,3-dihydroxy-5-iodobenzene monohydrate.

The model antigen, 2,4,6-tri-(*p*-azophenyl lactoside)-1,3-dihydroxy-5-iodobenzene-$^{125}I$ (3, X = *p*-$N_2C_6H_4OC_{12}H_{21}O_{11}$ = Lac) was prepared by coupling 4 mg of 1, labeled with 300,000 cpm of $^{125}I$, and 129 mg of freshly diazotized *p*-aminophenyl lactoside (carrier:hapten ratio of 1:4) at pH 9-10 and 4 C for 5 hrs; the rate of reaction was followed by the increase in absorbance at 456 nm, characteristic of triazophenylbenzene. As soon as the absorbance at 456 nm stabilized, the reaction was stopped by addition of *N* HCl to pH 5. The product which precipitated was recrystallized from 50% aqueous ethanol, yielding 5 mg of 3 (X = Lac).

Chicken anti-lactoside antibody (Schram, 1970) was precipitated by Compound 3 (X = Lac) following an ordinary antigen-antibody precipitation pattern (Figure 1). The serum precipitating anti-lactoside antibody level was 1.625 mg/ml, measured

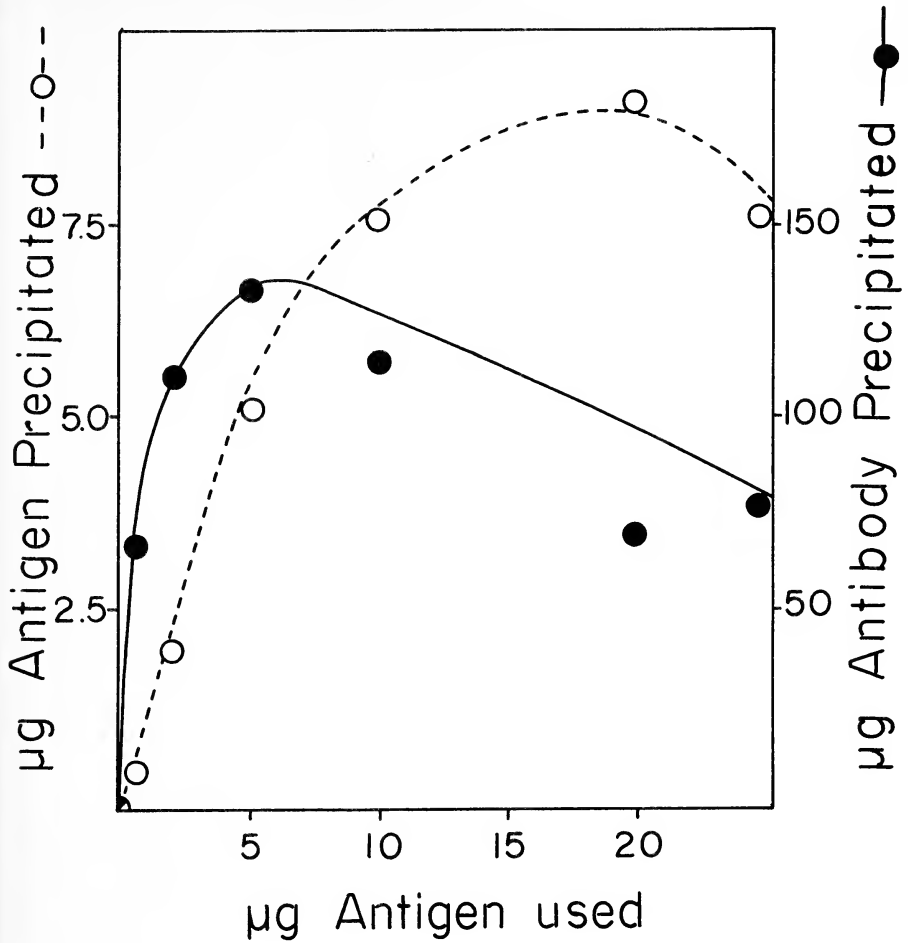


Figure 1. Precipitation of 3(X = Lac) by its specific antibody. The amount of 3(X = Lac) was calculated from the radioactivity of the precipitates, corrected for antigen controls without serum; the amount of antibody, by determination of the protein in the precipitates (Lowry, *et al.*, 1953), corrected for controls using the same levels of antigen and pre-immunization serum. Each point, thus, represents the results of the reaction between the amount of 3 (X = Lac) shown on the abscissa and 100 µl of chicken anti-lactoside antiserum (Schram, 1970), under conditions previously described (Schram, *et al.*, 1971).

by quantitative precipitation with an $^{125}$I-labeled bovine serum albumin (BSA) carrier, coupled to an average of 11 Lac groups/molecule of carrier. The total level of lactoside-binding antibody, measured by the Farr technique (Farr, 1958), was 1.77 mg/ml. Only 1.35 mg of the available antibody in 1 ml of serum was precipitated by Compound 3 (X = Lac). The shape of the precipitation curve,

however, indicated that all of the antibody which could be precipitated by Compound 3 (X = Lac) had been precipitated: a plateau was reached (Figure 1). Thus, only 83% of the available precipitating antibody was actually precipitated. Antibodies of varying avidities are probably present in the antiserum, but at first glance, it did not seem that avidity differences could be responsible for the difference between the amounts precipitated by $Lac_{11}$-BSA and by Compound 3 (X = Lac), since the Lac group was the same determinant in either case.

A probable explanation is based on the 3-dimensional restrictions imposed by the 1,3-dihydroxy-5-iodobenzene central structure. In Lac proteins, the Lac groups are distributed on the surface of the protein carrier wherever a tyrosyl or a histidyl residue is available (Schram, 1970). Since the protein carrier is much larger than the 1,3-dihydroxy-5-iodobenzene carrier, the distance between the Lac groups on the protein carrier is larger: approximately 50Å, assuming a random distribution on the surface of a BSA molecule with the shape of a 160Å x 35Å ellipsoid (Oncley, 1949).

The 6 diazo nitrogen atoms bound to the phenyl ring of 1 are isoplanar with the phenyl ring, because of the conjugation with the central ring's $\pi$ electrons. Although the 3 phenyl rings linking the diazo nitrogen atoms and the Lac groups are probably tilted (steric hindrance), free rotation would be restricted by conjugation; the lactosyl groups themselves, being in a *para* position with respect to the diazo nitrogen atoms, are restricted in rotation to the axis going through the acetal oxygen atom and the diazo nitrogen atoms, toward the center of the 1,3-dihydroxy-5-iodobenzene ring (Figure 2). The net result will be to limit the distance between neighboring lactosyl groups to approximately 15Å.

The antibody is complementary for the whole lactosyl group, but the phenyl ring and the atoms *para* to the lactosyl group still influence the binding to the antibody (Paktinat, 1976). In other words, the antibody polypeptide chains in the combining site envelop more than the lactosyl groups. The thickness of the polypeptide chain enveloping a lactosyl group may hinder the positioning of 2 other polypeptide chains around the other 2 lactosyl groups in Compound 3 (X = Lac). Thus, Compound 3 (X = Lac) will precipitate only that portion of the antibody population with shallower combining sites.

There is some evidence for this reasoning: the binding of a different sample of chicken anti-lactoside serum by a reference lactoside was inhibited by *p*-nitrophenyl galactoside (Schram, manuscript in preparation). However, the inhibition was weak, and its kinetics indicated that it involved a small proportion of the antibody population. This particular antibody population was still complementary to lactose, but with specificity for the galactoside portion of lactose only. Thus, the antiserum must have antibody populations with various depths of combining site.

In the serum used for this study, there were probably antibodies with combining sites of various depths, also. These populations may well be responsible for part of the avidity differences. Antibodies with shallower combining sites would have

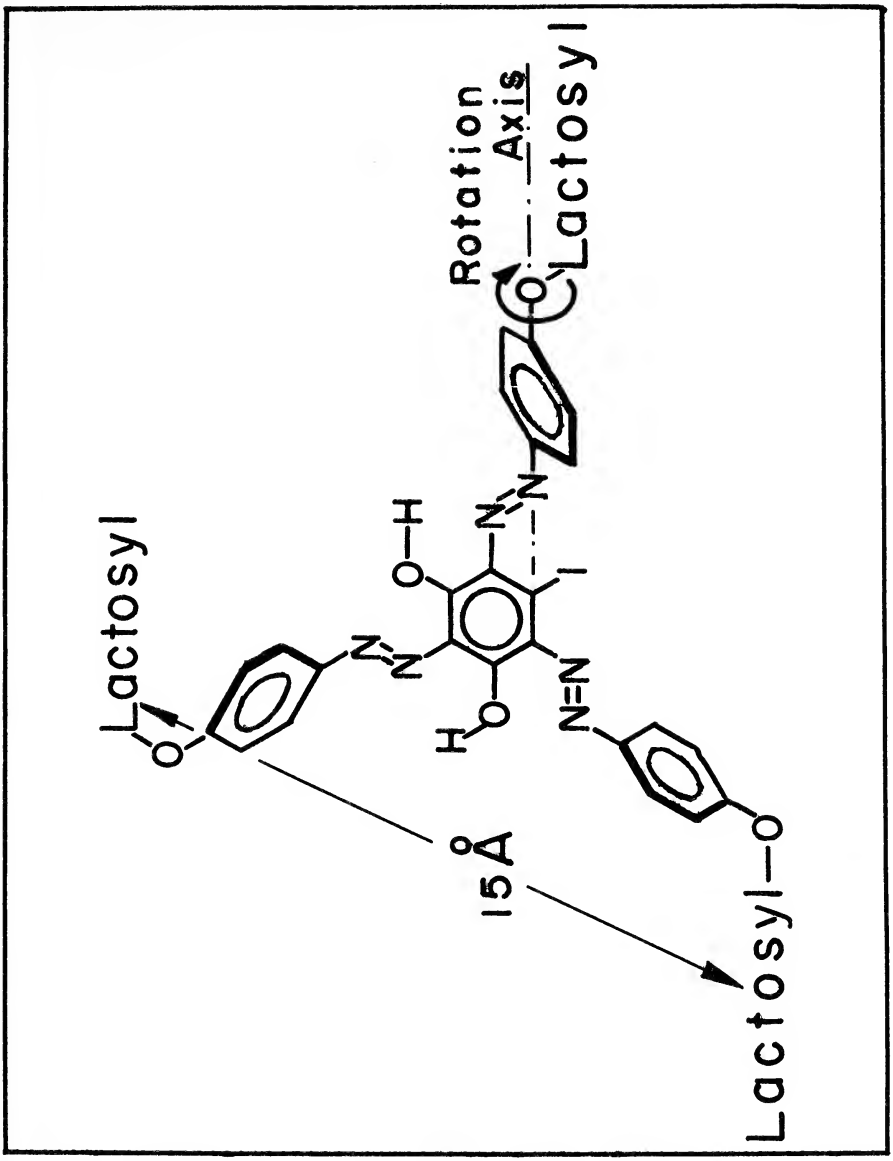


Figure 2. Spatial configuration of Compound 3 (X = Lac).

fewer potential hydrogen bonding groups; the binding energy for the antigen-antibody complex would be lower, and the complex would be more easily dissociated.

The 17% of the precipitating antibody which was not precipitated by Compound 3 (X = Lac) may represent a portion of the antibody population with the deepest combining site. To accommodate all of the antibodies present, it would

then be necessary to locate the Lac Groups further from the geometric center of Compound 3, perhaps by intercalation of several methylene groups between the Lac groups and the central phenyl ring.

Since any other haptenic group (X), easily bonded to Compound 1 could replace the Lac group of the example outlined, the carrier described is widely adaptable to those cases in which the determinant is chemically defined. We did not use purified antibody in this report, for the reason that purification by absorption and elution (which are equilibrium processes) tend to concentrate antibodies of low avidities in the eluates (Schram, *et al.*, 1971). The purified preparations, thus, do not represent the composition of the original serum.

## ACKNOWLEDGEMENTS

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# CORROSION STUDIES IN A WATER FLOOD OIL FIELD SYSTEM

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ABSTRACT

Corrosion studies of a water flood oil field system were undertaken. Experimental evidence indicated that a $CaCO_3$ and Fe containing film was forming on the interior surfaces of the mild steel pipe that handled production fluids. The injection water used for water flooding contained an insufficient amount of Ca to allow formation of a protective $CaCO_3$ film and was thus more corrosive than the production water.

## INTRODUCTION

The use of water flooding for the recovery of oil, after primary production techniques are exhausted, is now very common. Water flooding as a deliberately planned operation for secondary oil recovery had its origin in the Bradford and Allegany fields of Pennsylvania and New York, in 1920 (Muskat, 1949). This type of secondary recovery operation involves the forcing of water, under pressure, into the ground via a number of injection wells to force out any remaining oil not removed by a primary recovery operation. Permeation of this injection water through the surrounding formation results in the formation of an oil bank ahead of the advancing water which is subsequently removed by a producing well.

In water flood operations, attention must be given to the character and treatment of the injection water in order to prevent corrosion and/or plugging of the formation. Because of the large volumes of water used in this type of recovery, the unit cost of treatment/barrel of water injected must be small. In many instances the source of the injection water used is the local producing oil wells. This water is usually very corrosive because of its high NaCl content and the presence of either $O_2$, $H_2S$, or $CO_2$, or a combination of these gases.

This study concerns a water flood oil recovery system of more than 100 producing shallow oil wells. The water flood operation is necessary to obtain the oil from the sand formations. The injection water used is a mixture of water from large water wells outside the oil field and water that has been separated from production fluids. For this discussion, injection water obtained from the external water wells will be referred to as Well Injection (W.I.) water. Water from these wells is transported via a common handling system to the oil field where it is

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then pumped into the ground at various sites. Water that has been separated from combined production fluids of all the producing wells will be referred to as Composite Production (C.P.) water. Four other water samples, taken from 4 different sites in the oil field, were also studied. They will be referred to as Production Water P-1, P-2, P-3, and P-4.

When this water flood operation was started approximately 10 years ago, the C.P. water contained almost all natural occuring formation water. This was also true for Production Water samples taken at the same location samples P-1, P-2, P-3, and P-4 were taken. Very little corrosion was observed in the production fluid handling system. However, significant corrosion problems were observed in the W.I. handling system. These problems were solved by the use of cement lined tubing. During the past 10 years, the composition of the production fluids in some portions of the field have become more like that of the W.I. water.

This study was initiated to determine what future corrosion problems might occur in this field, as the composition of the formation water approaches that of the W.I. water.

## METHODS AND RESULTS

Corrosion rates for mild steel (No. 1018) in samples of W.I., C.P., 50-50 mixture of W.I. and C.P., and P-1, P-2, P-3, and P-4 water were made using direct weight loss measurements and corrosion rate meter measurements. The steel specimens used were cylindrical with a surface area of 8.74 $cm^2$. All specimens were sanded lightly with steel wool, polished with an emory cloth, washed in hot benzene, rinsedi in acetone, and dried in a vacuum dessicator prior to use.

For direct weight loss measurements, each specimen was weighed using an analytical balance, and then placed in the corrosion test solution for 24, 48, or 72 hrs. The test solution was closed to the atmosphere and stirred using a Sargent magnetic stirrer set on a scale reading of 4. When a specimen was removed from the test solution, any corrosion products remaining on the surface of the specimen were removed using a hard gum eraser. The specimen was then washed in hot benzene, rinsed in acetone, and dried in a dessicator. The steel specimen was then weighed and the weight loss determined by comparison with the initial weight. Using this weight loss data, the corrosion rates in mils/yr (mpy) were calculated using the equation

$$\text{mpy} = \frac{3440W}{DAT}$$

where W = weight loss in mg
D = density of the test specimen in $g/cm^3$
A = area of the test specimen in $cm^2$
T = time of exposure in hrs
1 mil = 0.001 in

Corrosion rate meter measurements were made using a Petrolite Corrosion Rate Meter, Model M-103. This instrument utilized 3 electrodes; a reference, auxiliary, and test electrode. For this instrument, the reference and auxiliary electrodes have the same composition as the test electrode. The instrument electronics are designed to utilize the equation developed by Stern and Geary (1957)

$$\frac{\Delta E_{app}}{\Delta I} = \frac{B_a B_c}{2.3(I_{corr})(B_a + B_c)}$$

where $B_a$ and $B_c$ are the Tafel slopes of the anodic and cathodic reactions, respectively, and

$\Delta E_{app}$ = 10 mV for this instrument

$\Delta I$ = change in current as a result of $\Delta E_{app}$

$I_{corr}$ = corrosion current.

The instrument automatically converts $I_{corr}$ to mils/yr.

The corrosion rates obtained using these methods are listed in Table 1.

TABLE 1

Corrosion Rates of Different Waters.

| Water Type | Time (hrs) | Weight Loss (mpy) | Average (mpy) | Corrosion Rate Meter (mpy) | Average (mpy) |
|---|---|---|---|---|---|
| W.I. | 24 | 36.3 | | 34.0 | |
| | 48 | 37.8 | 36.3 | 35.0 | 33.3 |
| | 72 | 34.7 | | 31.0 | |
| C.P. | 24 | 22.7 | | 22.0 | |
| | 48 | 18.5 | 21.4 | 20.5 | 22.0 |
| | 72 | 23.0 | | 21.5 | |
| 50-50 Mixture | 24 | 14.9 | | | |
| of W.I. | 48 | 14.7 | 14.3 | | |
| and C.P. | 72 | 13.3 | | | |
| Production | 24 | 19.7 | | | |
| Water, P-1 | 48 | 16.0 | 18.0 | | |
| | 72 | 18.4 | | | |
| Production | 24 | 14.3 | | | |
| Water, P-2 | 48 | 13.4 | 13.9 | | |
| | 72 | 14.1 | | | |
| Production | 24 | 22.3 | | | |
| Water, P-3 | 48 | 24.3 | 24.8 | | |
| | 72 | 27.7 | | | |
| Production | 24 | 19.9 | | | |
| Water, P-4 | 48 | 16.8 | 17.5 | | |
| | 72 | 15.7 | | | |

All water samples were analyzed by a private testing laboratory. These results are given in Table 2. It is well know that in water systems, $Cl^-$ and $SO_4^{-4}$ enhance corrosion while $Ca^{+2}$, $Mg^{+2}$, and $HCO_3^-$ ions act as mild corrosion inhibitors. In Table 2, it is easy to see that natural formation water is high in $Cl^-$, $SO_4^{-2}$, $Ca^{+2}$, and $Mg^{+2}$, and the $HCO_3^-$ level in the W.I. water is slightly higher than that of the natural formation water.

TABLE 2

Water Analysis

Well Injection Water

Total Dissolved Solids, 14,976 mg/l; Resistivity, 0.4233 Ω-m at 78 F; Specific Gravity, 1.0128 at 60 F; pH, 8.8 at 79 F

| Constituents | meq/l | mg/l | Constituents | meq/l | mg/l |
|---|---|---|---|---|---|
| Sodium | 242.11 | 5,566.0 | Chloride | 211.55 | 7,499.0 |
| Calcium | 1.92 | 38.5 | Bicarbonate | 11.23 | 685.0 |
| Magnesium | 3.06 | 37.2 | Sulfate | 22.82 | 1,096.0 |
| Iron | 0.16 | 4.5 | Carbonate | 1.65 | 49.5 |
| Barium | 0.00 | 0.0 | Hydroxide | 0.00 | 0.0 |

Composite Production Water

Total Dissolved Solids, 81,551 mg/l; Resistivity, 0.0885 Ω-m at 79 F; Specific Gravity, 1.0689 at 60 F; pH, 7.55 at 79 F

| Constituents | meq/l | mg/l | Constituents | meq/l | mg/l |
|---|---|---|---|---|---|
| Sodium | 1,259.12 | 28,947.00 | Chloride | 1,300.99 | 46,118.0 |
| Calcium | 86.98 | 1,743.00 | Bicarbonate | 4.51 | 275.0 |
| Magnesium | 41.79 | 508.00 | Sulfate | 82.43 | 3.959.0 |
| Iron | 0.04 | 1.25 | Carbonate | 0.00 | 0.0 |
| Barium | 0.00 | 0.00 | Hydroxide | 0.00 | 0.0 |

Production Water, P-1

Total Dissolved Solids, 39,233 mg/l; Resistivity, 0.20000 Ω-m at 75 F; Specific Gravity, 1.0248 at 60 F; pH, 7.35

| Constituents | meq/l | mg/l | Constituents | meq/l | mg/l |
|---|---|---|---|---|---|
| Sodium and Potassium | 492.9 | 11,337.0 | Chloride | 456.7 | 16,194.0 |
| Calcium | 58.6 | 1,174.0 | Bicarbonate | 10.2 | 620.0 |
| Magnesium | 13.8 | 168.0 | Sulfate | 98.4 | 4,730.0. |
| Iron | Trace | Trace | Carbonate | -- | -- |
| Barium | 0.0 | 0.0 | Hydroxide | -- | -- |

Table 2 (Continued)

Production Water, P-2

Total Dissolved Solids, 125,962 mg/l; Resistivity, 0.062 Ω-m at 75 F; Specific Gravity, 1.0911 at 60 F; pH, 7.3

| Constituents | meq/l | mg/l | Constituents | meq/l | mg/l |
|---|---|---|---|---|---|
| Sodium and Potassium | 1,934.6 | 44,496.0 | Chloride | 2,052.6 | 72,786 |
| Calcium | 117.1 | 2,347.0 | Bicarbonate | 8.0 | 488 |
| Magnesium | 95.8 | 1,165.0 | Sulfate | 86.9 | 4,180 |
| Iron | Trace | Trace | Carbonate | -- | -- |
| Barium | 0.0 | 0.0 | Hydroxide | -- | -- |

Production Water, P-3

Total Dissolved Solids, 57,215 mg/l; Resistivity, 0.125 Ω-m at 75 F; Specific Gravity, 1.0412 at 60 F; pH, 7.80

| Constituents | meq/l | mg/l | Constituents | meq/l | mg/l |
|---|---|---|---|---|---|
| Sodium and Potassium | 865.8 | 19,913.0 | Chloride | 859.3 | 30,473 |
| Calcium | 80.9 | 1,622.0 | Bicarbonate | 3.2 | 195 |
| Magnesium | 16.0 | 194.0 | Sulfate | 100.2 | 4,818 |
| Iron | Trace | Trace | Carbonate | -- | -- |
| Barium | 0.0 | 0.0 | Hydroxide | -- | -- |

Production Water, P-4

Total Dissolved Solids, 131,548 mg/l; Resistivity, 0.061 Ω-m at 75 F; Specific Gravity, 1.0934 at 60 F; pH, 6.70

| Constituents | meq/l | mg/l | Constituents | meq/l | mg/l |
|---|---|---|---|---|---|
| Sodium and Potassium | 2,068.4 | 47,573.0 | Chloride | 2,160.6 | 76,617 |
| Calcium | 149.1 | 2,988.0 | Bicarbonate | 7.0 | 429 |
| Magnesium | 25.6 | 311.0 | Sulfate | 75.5 | 3,630 |
| Iron | Trace | Trace | Carbonate | -- | -- |
| Barium | 0.0 | 0.0 | Hydroxide | -- | -- |

Since the ion concentrations varied in the different waters, it was thought that these various concentrations might have a noticeable effect on the corrosion rate. Two hundred ml of C.P. water was evaporated and the remaining salts added to 200 ml of W.I. water. The corrosion rate of mild steel in the W.I. water decreased from 36 mpy to 18 mpy after addition of these salts. It was then decided to vary the different ion concentrations selectively and to measure the resulting corrosion rates.

As shown in Table 1, P-2 was the least corrosive water. $Ca^{+2}$, $Mg^{+2}$, and $Cl^-$ ions were added to 3 samples of W.I. water to make the respective ion concentration equal to that of P-2, and the corrosion rates of mild steel in these samples measured. Table 3 gives the results of these measurements. The $Ca^{+2}$ ions and $Cl^-$ ions caused a major decrease in the corrosion rate. No $HCO_3^-$ ions were added to the W.I. water since it has a high $HCO_3^-$ concentration. So by varying the concentrations of the $Ca^{+2}$ and $Cl^-$ ions, the corrosive properties of the W.I. water could be made similar to that of the production waters.

TABLE 3

Corrosion Rates of Well Injection Water with Ions Added.

*W.I. water with $CaCl_2$ added:*

| Time (hrs) | Corrosion Rate (mpy) | Average (mpy) |
|---|---|---|
| 24 | 7.99 | |
| 48 | 7.58 | 7.31 |
| 72 | 6.35 | |

*W.I. water with NaCl added:*

| Time (hrs) | Corrosion Rate (mpy) | Average (mpy) |
|---|---|---|
| 24 | 15.8 | |
| 48 | 21.0 | 18.7 |
| 72 | 19.3 | |

*W.I. water with $MgCl_2$ added:*

| Time (hrs) | Corrosion Rate (mpy) | Average (mpy) |
|---|---|---|
| 24 | 36.0 | |
| 48 | 38.2 | 35.9 |
| 72 | 33.7 | |

Calcium carbonate is well known for its ability to act as a cathodic corrosion inhibitor (Bregman, 1963). $CaCO_3$ deposits on a metal result in an ennoblement of the metal for the cathodic reaction (thus it is called a cathodic inhibitor). Anodic and cathodic potentiostatic polarization curves for mild steel in samples of W.I., C.P. and 50-50 mixtures of W.I.-C.P. water were made. An Elron potentiostat (Model CHP-1) was used as a constant potential source and a Keithley electrometer (Model 602) was used to monitor the potential change versus a saturated calomel electrode. An ammeter, built into the potentiostat, was used to record the current. The electrolytic solution was at room temperature and stirred using a Sargent magnetic stirrer set at 5. Current readings were recorded at each potential after they had maintained a constant value for 5 min. The polarization curves obtained are shown in Figure 1. The cathodic portions of the C.P. and 50-50 mixture indicate an ennoblement of the steel, with the 50-50 mixture producing the greatest effect.

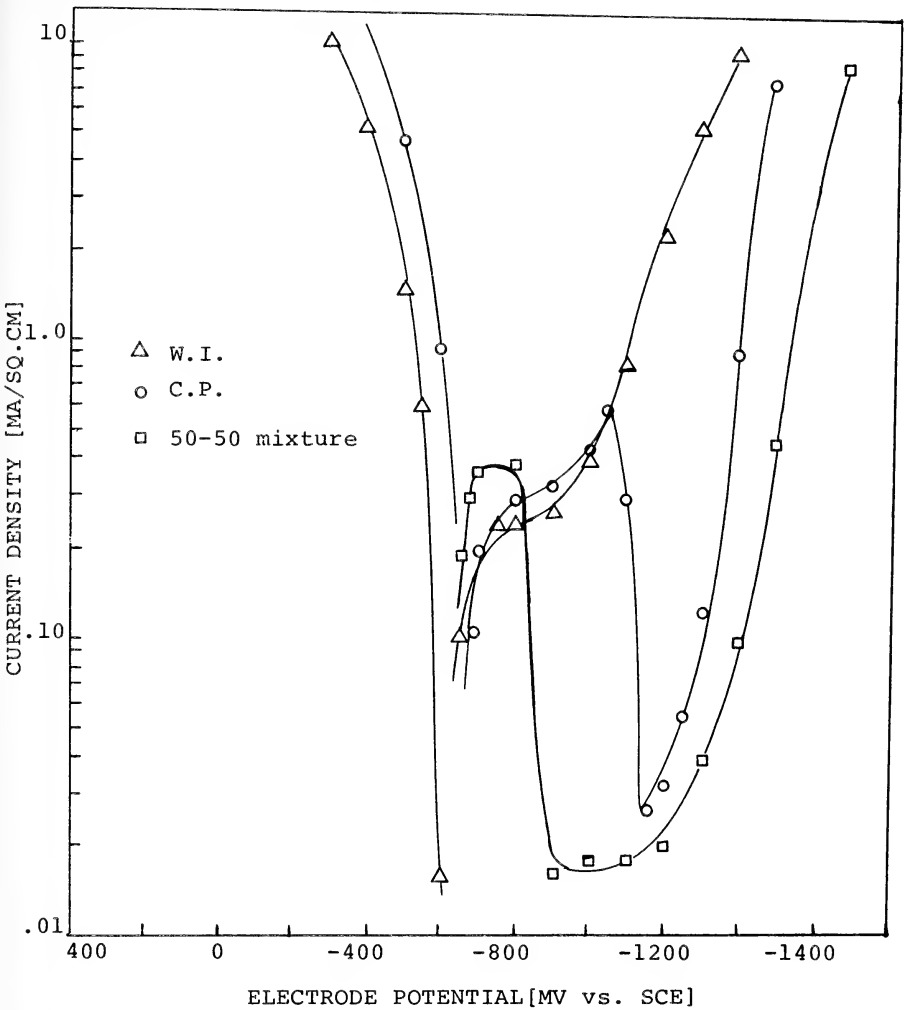


Figure 1. Polarization curves for mild steel.

Film analyses were made on mild steel specimens exposed to W.I. water with $CaCl_2$ added to it. The film was dissolved from the specimen by immersing it in 10% HCl. The solution was then analyzed for $Ca^{+2}$ using a Beckman, Model 979, atomic absorption system. Assuming that all the $Ca^{+2}$ was due to $CaCO_3$, the film contained approximately 85% by weight $CaCO_3$. When the specimen was placed in the HCl solution, vigorous bubbling accompanied the decomposition of the film (probably $CO_2$).

As mentioned above, $CaCO_3$ is an effective corrosion inhibitor in some systems. A thin, adherent, protective film of $CaCO_3$ is effective in some types of water treatment. The equilibrium between $HCO_3^-$ and $CO_3^{-2}$ ions is represented by the following:

$$2HCO_3^- \rightleftarrows CO_3^{-2} + CO_2 + H_2O.$$

A possible mechanism for the corrosion observed in this particular study is as follows:

at the anodic sites: $Fe \rightarrow Fe^{+2} + 2e^-$

$Fe^{+2} + 2OH^-$ (from cathodic sites) $\rightleftarrows Fe(OH)_2$

at the cathodic sites:

in the absence of $O_2$: $2H_2O + 2e^- \rightarrow 2OH^- + H_2$

in the presence of $O_2$: $\frac{1}{2}O_2 + H_2O + 2e^- \rightarrow 2OH^-$

$Ca^{+2} + 2OH^- \rightleftarrows Ca(OH)_2$

$Ca(OH)_2 + HCO_3^- \rightleftarrows CaCO_3 + H_2O + OH^-$

or

$HCO_3^- + OH^- \rightleftarrows H_2O + CO_3^{-2}$

$Ca^{+2} + CO_3^{-2} \rightleftarrows CaCO_3$.

Evans (1960) points out that a protective film of $CaCO_3$ is only formed from bicarbonate waters if the $CO_2$ content is limited to the amount needed to stabilize the $Ca(HCO_3)_2$ that is present. In this manner, the smallest increase in pH suffices to render the liquid next to the metal supersaturated with $CaCO_3$.

A saturation point of $HCO_3^-$ and $Ca^{+2}$ must be maintained for cathodic inhibition to occur. Evans (1960) and Bregman (1963) have stated that formation of a $CaCO_3$ film will occur even if the bulk solution is not at its saturation point. This is because the $Ca^{+2}$ ions will migrate to the negative cathodic sites, where the pH is higher than that of the bulk solution due to formation of $OH^-$ ions. This results in formation of a protective $CaCO_3$ film.

The control of pH is an essential part of the water treatment. A pH of 6.0 to 6.5 is the minimum value that must be maintained, since below this value corrosion proceeds rapidly. Also a maximum value of 7.5 to 8.0 must not be exceeded, since at higher pHs scaling will occur. In practice, for better corrosion inhibition the pH is kept as high as possible without having scaling occur. Langeleir (NACE, 1971) has devised an index which is the difference between the actual pH value of water and the pH value reached when water is brought into equilibrium with $CaCO_3$. It indicates when the possibility of a $CaCO_3$ protective film will be formed. Finally, other suspended solids or deposits present in the system can act as a seeding material for the precipitation of the $CaCO_3$.

A graph was constructed of the corrosion rate of mild steel versus the product of the $Ca^{+2}$ ion and the square of the $HCO_3^-$ ion concentrations in meq/l. This graph is shown in Figure 2. An important trend can be seen in this graph. As the product of the ion concentration increases, the corrosion rate decreases. W.I. water has the greatest corrosion rate while having the smallest concentration product value. The production waters containing the most original formation water contains the highest $Ca^{+2}$ ion concentration. W.I. water has only small amounts of

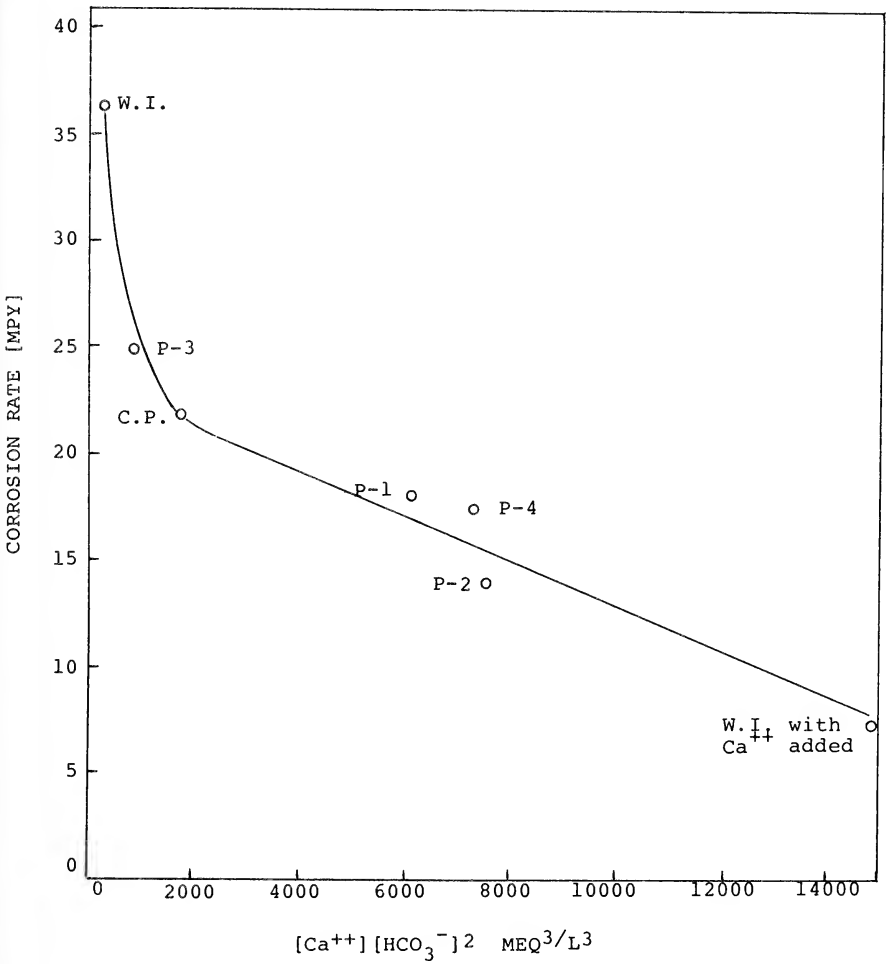


Figure 2. Effect of $Ca^{+2}$ and $HCO_3^-$ ions on corrosion rate.

$Ca^{+2}$ but is rich in $HCO_3^-$ ions. Thus, by adding $Ca^{+2}$ to W.I. water, its corrosion rate should decrease significantly. The $HCO_3^-$ concentration of W.I. is very high and thus the equilibrium between $HCO_3^-$ and $CO_3^{-2}$, described above, is shifted to the right when $Ca^{+2}$ ions are added to the system. This favors the formation of $CaCO_3$.

As shown in Table 3, the NaCl that was added to the W.I. water caused the corrosion rate to decrease. Foley (1970) describes the role $O_2$ and $Cl^-$ ions play in corrosion processes. He states that $O_2$ acts as a cathode depolarizer, causing $Fe^{+2}$ to go into solution, oxidizes the dissolved $Fe(OH)_2$ to $Fe(OH)_3$, and restores an oxide film. In the absence of $O_2$, saturated NaCl solutions are essentially non-corrosive to Type 1020 steel. In many studies it has been amply shown that in

neutral solutions and at temperatures up to about 80 C, the $O_2$ effect is important. A good discussion on the effects of $O_2$ on metal dissolution rates is given by Bockris and Reddy (1970). The fact that concentration effect and $O_2$ availability are interrelated has been observed by many investigators. As $Cl^-$ ion concentration increases, the solubility of $O_2$ decreases, thus decreasing the corrosion rate. Therefore, the reduction in corrosion rate when NaCl was added to W.I. water was probably due to the decreased solubility of $O_2$ (even though it may be present in low concentrations) in the water.

Alexander and Foley (1975) present a good discussion on the anion ($SO_4^{-2}$, $Cl^-$, $Br^-$, and $I^-$) dependence of the activation energy for Fe corrosion. The mild steel used in this study was approximately 99% Fe.

Also, from Table 3 it can be seen that the addition of $Mg^{+2}$ ions had little effect on the corrosion rate. This is because $MgCO_3$ is much more soluble in water than $CaCO_3$. Therefore, very little protective $MgCO_3$ would be available to form a protective film on Fe.

When discussing ion concentrations present in the waters, it is not completely advantageous to look at one ion concentration by itself. The corrosive properties of waters are due to a composite of all ions and dissolved gases present in them. There exists no defined points where one ion concentration may override the effect of another ion present. This sometimes leads to a complicated system which is often difficult to explain. The presence or absence of one ion may or may not enhance the inhibition properties of the other. This is also true for the gases that may or may not be present. Also, the degree of effectiveness of the ions as inhibitors must be taken into account. Therefore, we must always keep in mind the total picture when discussing these corrosive waters.

## CONCLUSIONS

Corrosion studies of a water flood oil recovery system were undertaken. Past field experiences had indicated that portions of the oil field handling mainly W.I. water were experiencing corrosion problems. Mild steel in contact with production waters did not undergo serious corrosion.

Our data indicates that a thin, protective $CaCO_3$ film is being formed on the steel that handles production fluids. In the W.I. system, a low $Ca^{+2}$ concentration prevents formation of this film. As the ratio between W.I. water and the original formation water increases in the future, the ratio of the concentration of $Ca^{+2}$ and $HCO_3^-$ ions will decrease. This will cause a decrease in the amount of shift of the $HCO_3^-$–$CO_3^{-2}$ equilibrium to the right, thus decreasing the amount of $CaCO_3$ formed at the metal surface. Thus while the current production waters cause relatively low corrosion rates on mild steel, this will change as more W.I. water enters the formation.

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# THE SALINITY AND TEMPERATURE TOLERANCE AND THE GROWTH OF *MACROBRACHIUM OHIONE* (SMITH) 1874 REARED IN LABORATORY TANKS[1]

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ABSTRACT

The salinity and temperature tolerances and the growth of *Macrobrachium ohione* was observed in laboratory tanks at the National Marine Fisheries Service, Galveston, Texas.

Salinities over 13 ppt and temperatures over 31.5 C increased mortality, especially during molting periods.

In an effort to determine maximum size of *M. ohione* the relationship between initial body length ($l_t$) and terminal body length ($L_{t+T}$) was plotted and the Walford line was found $L_{t+T} = 96.79431\,(1-e^{-2.40192}) + e^{-2.40192} l_t$. The calculated maximum body length, carapace length, and body weight were 96.79 mm, 28.23 mm, and 11.47 g, respectively.

## INTRODUCTION

The continuing population growth of the world has initiated a strong demand to increase agricultural as well as aquacultural products and to examine the economic feasibility of culturing many invertebrate fishery resources. River shrimp (*Macrobrachium*) is one of the prospective groups of protein foods which is receiving much attention and many researchers are studying intensive culture problems. Salinity, temperature, and growth on the Malaysian giant shrimp, *M. rosenbergii,* as it relates to the aquaculture and larval development have been reported by Fujimura (1966, 1970), Lewis (1962, 1965, 1966), Ling (1962, 1967a, 1967b, 1971), Ling and Merican (1961), Uno and Kwon (1969), and Wickins (1969, 1972). Larval development of *M. acanthurus* and *M. carcinus* has been studied by Choudhury (1970, 1971a, 1971b, 1971c). Ingle and Edward (1960) have noted the possibility of artificially culturing them. Ecology, larval development, and aquaculture problems relating to salinity, temperature, and breeding cycle of *M. nipponense* were reported by Chung (1972), Kwon and Uno (1969), and Uno (1971), respectively.

Four species of Macrobrachium (*M. ohione, M. olfersi, M. acanthurus,* and *M. carcinus*) in Texas are reported by Hedgpeth (1947, 1949) and Reimer and Trudeau (1975). *Macrobrachium ohione* is the most common and smallest of the 4 species.

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It is found along the coastal line and inland waters of the United States from Virginia (Hobbs and Massmann, 1952) to Central Texas (Holthuis, 1952).

Dugan (1971, 1972) reported on salinity tolerances in the larval stages of this species and Reimer, *et al.*, (1974) have discussed the general ecology of *M. ohione* collected from Galveston Bay, in natural environments. Reimer, *et al.*, (1974) suggest that juvenile and adult *M. ohione* live in the freshwater and avoid salinities above 15 ppt. Temperatures near 30 C represent their upper tolerance level.

This work was done in 2 parts. The 1st experiment was for evaluating salinity and temperature effects on survival and ran for 30 days. The 2nd experiment was concerned with growth in freshwater and ran for 30 days.

## MATERIALS AND METHODS

*M. ohione* were collected from the San Bernard River, Brazoria County, Texas.

The shrimp were held in aged tap water until the beginning of each experiment. Forty-eight shrimp of only 1 species were transported in an ice cooler (60 x 35 x 35 cm) with aeration on 22 June 1973 for temperature and salinity experiments. An additional 30 shrimp were transported, as in the above method, on 15 July 1973 for growth experiments. All groups of shrimp were fed an experimental hard pellet-type food of Louisiana State University, about 5% of the total body weight of the group every day. A distinction between sex was not made.

*Salinity – Temperature*

Shrimp changed from fresh to salt water were first allowed to acclimate for 6 hrs in 2 ppt salinity. Each group of shrimp to be held at higher salinities was allowed to acclimate for 6 hrs at each of the test salinities (4, 6, 10, 13 ppt) until they reached the specific salinity they were to be held at. Salinity levels are continuously fluctuating in estuaries with diurnal tides. The median of 6 hrs-salinity-acclimation was arbituary chosen. Due to the variable chemical characteristics of natural seawater, synthetic sea salt, Instant Ocean, was used in the experiments. This type of solution was found adequate for penaeid shrimp culture by Cook and Murphy (1969) and Mock and Murphy (1971). Salinities were regulated by use of an American Optical Goldberg T/C refrectometer. The salt solution was aged for at least 24 hrs in a 500 gal (1.89 $m^3$) tank before the experiment began. Eight shrimp were kept in each of 6 experimental tanks, 80 x 50 x 45 cm in dimension with each tank containing approximately 150 l of water. A biological filter using oyster shells was connected with each tank so that the water was continually circulated. In addition, water was circulated in each tank by air dispensed by a MillionAire air stone (Figure 1). Dissolved oxygen levels were not measured but were assumed to be adequate.

The experimental tanks were located in a green house that was roofed with gray corrugated fiber glass sheeting. Each tank was covered with fiber glass sheeting to prevent the shrimp from jumping out of the tank during the experiment.

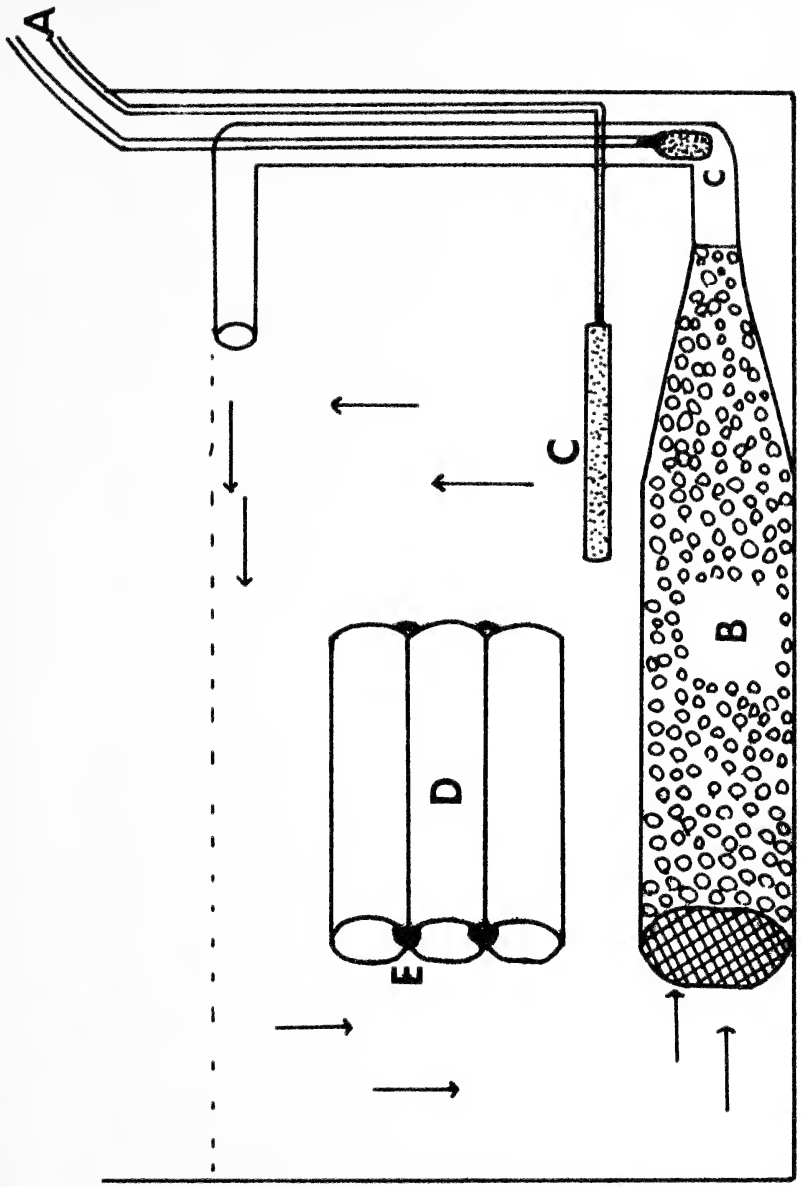


Figure 1. Experimental tank with biological filteration and air circulation (A, air tube; B, shell tube; C, MillionAire air stone, c, air stone; D, PVC pipe; E, lead combiner).

Water was not changed during experiments, but distilled water was added every day to correct salinity changes as a result of evaporation.

Temperature was measured with a Fisher's 15-043A thermometer twice daily at 8 am and 5 pm. The temperature in each tank was allowed to fluctuate with atmospheric temperature.

*Growth*

Since good survival was found to occur in freshwater, this medium was used for the growth study. The tank and filter system used in the first experiment were also used here. Temperature was allowed to fluctuate with atmospheric conditions.

Shrimp were measured according to total body length (tip of rostrum to distal end of telson), carapace length (posterior margin of orbit to the dorsal posterior margin of carapace) and body weight. Measurements were to the nearest 0.1 mm for body length, 0.01 mm for carapace length, and were made with caliper. Length measurements were taken before and after experiments. For weighing the shrimp, individuals were removed from the tank, carefully wiped with paper towel to remove excess water, and placed on the balance.

The body length obtained after 30 days was plotted against that of the initial length to find maximum length to be expected in ponds. A Walford (1946) type line was used for this determination. The instantaneous growth rate using body length was determined based on the procedures by Gulland (1969).

Estimate of daily survival rate was calculated by $S = N_t/N_o$ (Ricker, 1975) assuming constant survival rate during experimental days where S is survival rate, $N_o$ is initial population size, and $N_t$ is population size time t. Instantaneous mortality rate (Z) was calculated by $e^{-zt}=N_t/N_o$ assuming mortality rate is constant during experimental days.

## RESULTS

*Salinity – Temperature*

The % survival in each experimental tank after 30 days was as follows: 87.5%, in aged tap water; 84.1% in 2 ppt; 82.5% in 4 ppt; 80.8% in 6 ppt; 72.8% in 10 ppt and 52.0% in 13 ppt. An analysis of variance was run to determine if these survival rates were significantly different (Table 1).

TABLE 1

Analysis of variance of survival rate of test animals in 6 salinities and Tukey's honestly significant difference test based on residual (mean not spanned by the line is significantly different).

| | ANOVA | | | |
|---|---|---|---|---|
| Source | d.f. | SS | MS | F |
| Salinities | 5 | 25,465.63 | 5,093.13 | 12.75** |
| Survival rate in salinities | 174 | 69,497.77 | 399.41 | |
| Total | 179 | 94,693.40 | | |

| | Tukey's Test | | | | | |
|---|---|---|---|---|---|---|
| Salinity (ppt) | 0.0 | 2.0 | 4.0 | 6.0 | 9.0 | 13.0 |
| Mean survival (%) | 87.5 | 84.1 | 82.5 | 80.8 | 72.8 | 52.0 |

**Significance at 1%

The total survival rate of the 6 experimental groups was significantly different at 1% level. To determine which group or groups were significantly different, a Tukey's honestly significant difference test was run. The survival rate of those shrimp in 13 ppt salinity was significantly different from that of other salinities at the 1% level (Table 1). The relationship between salinity (s) and survival rate (S) was calculated with $S = 94.14559s^{-.1515}$ (Figure 2).

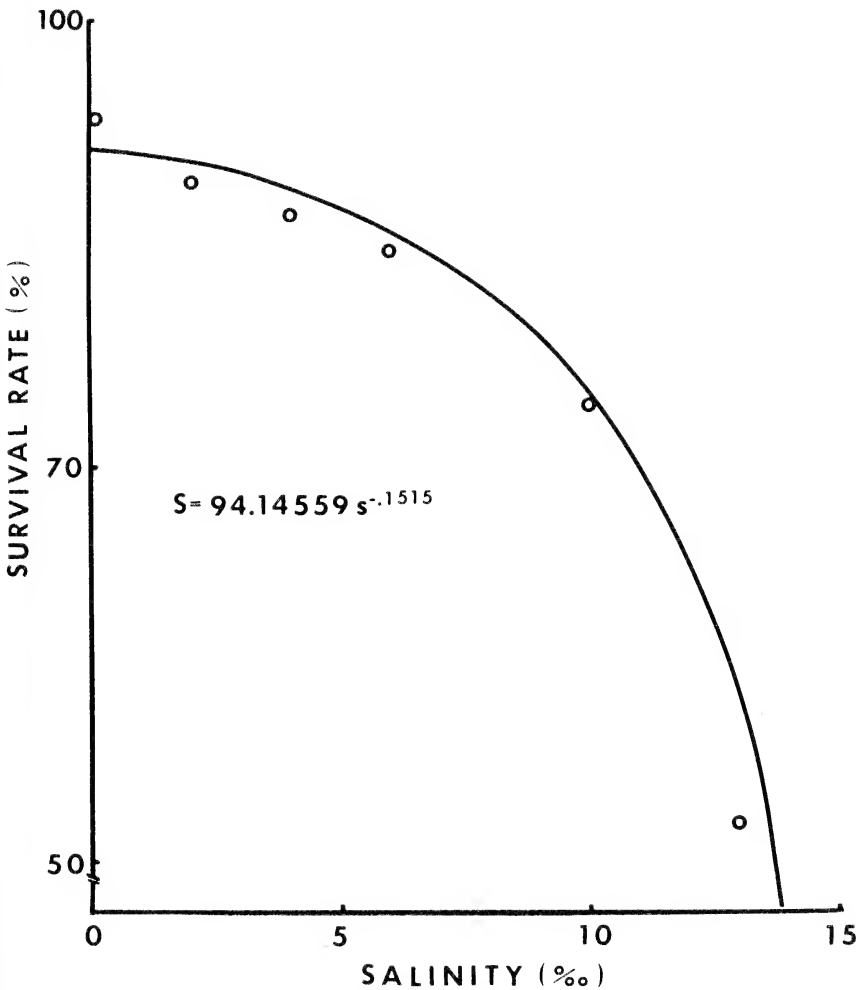


Figure 2. Relationship between salinity (s) and survival rate (S) of *M. ohione*.

Most deaths occurred during ecdysis at temperatures of 31.5 C and greater. The relationship between temperature (T) and % of mortality (Z) was calculated with $Z = (0.53604 \times 10^{-34})T^{23.7949}$. Afternoon temperatures ranged from 28.5 C to 32.9 C and morning temperatures from 25.7 C to 28.7 C (Figure 3). All shrimp deaths were reported in the afternoon and when temperatures were above 31.5 C. In every case where death was recorded some shrimp were observed to be molting.

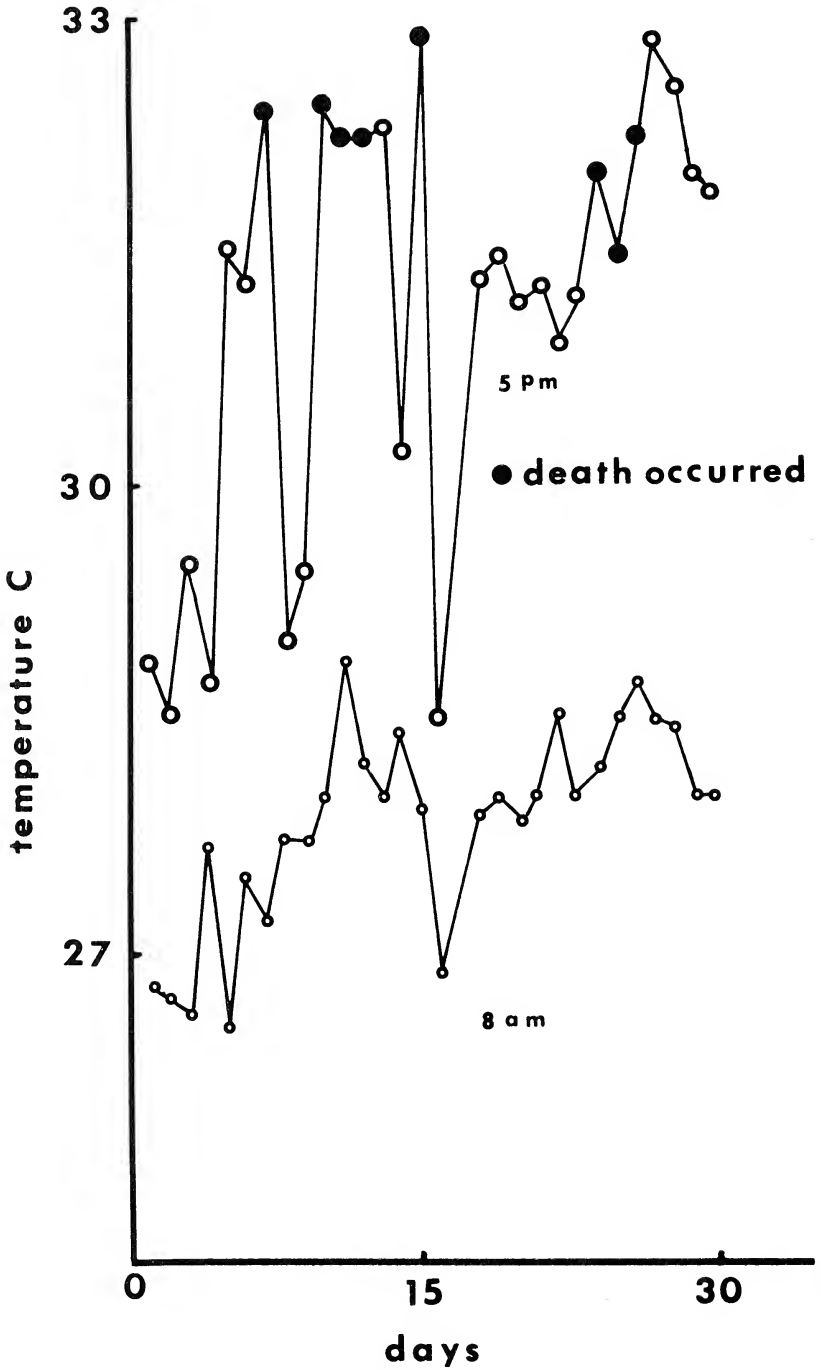


Figure 3. Temperature and death during experiment days of *M. ohione*.

*Growth*

The relationship between initial ($l_t$) and terminal ($L_{t+T}$) body length was plotted using the Walford type formula (Walford, 1946), $L_{t+T} = 96.79431\,(1-e^{-2.40192}) + e^{-2.40192} l_t$ (Figure 4). Calculated maximum carapace length was 28.23 mm.

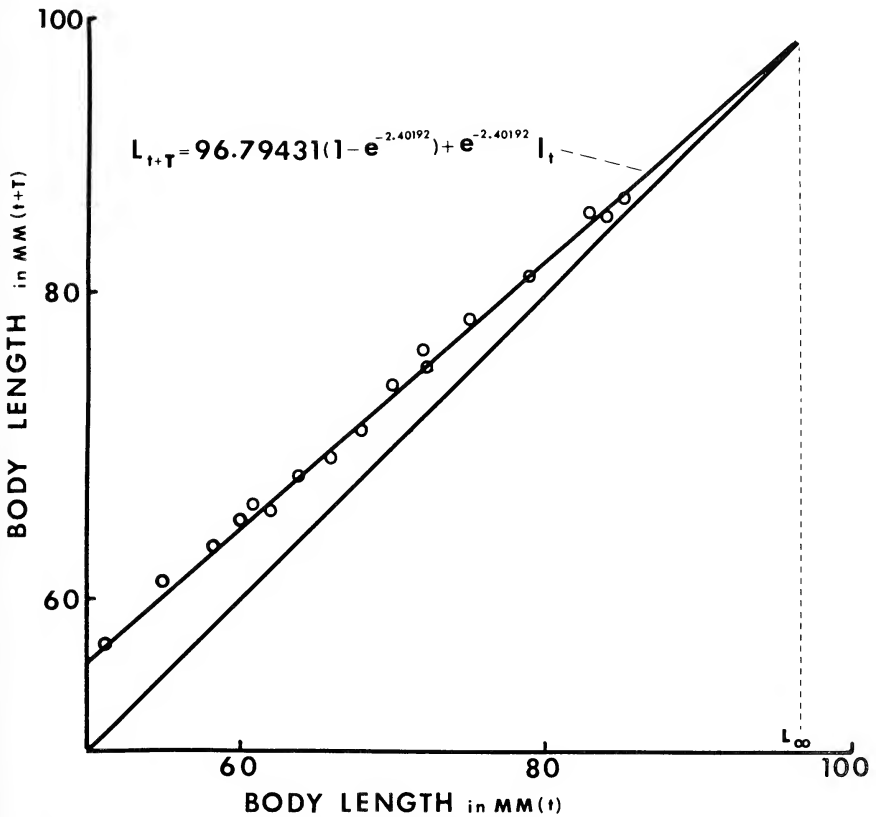


Figure 4. Relationship between *M. ohione* body length before ($l_t$) and after experiments ($L_{t+T}$).

Relationship between carapace length (C) and body length (L) was presented with $L = 10.49223 + 3.05742\,C$ (Figure 5).

Relationship between body length (L) and body weight (W) was calculated to be $W = (0.18625 \times 10^{-6})L^{3.9225}$ (Figure 6).

## DISCUSSION

*Salinity – Temperature*

Laboratory experiments showed that differences in survival rates of *M. ohione* in freshwater and at salinities of 2, 4, 6 or 10 ppt were not significant at 1% level. However, the mortality of shrimp at 13 ppt was significantly different from freshwater as well as the other salinities (Table 1). Hobbs and Massmann (1952) have

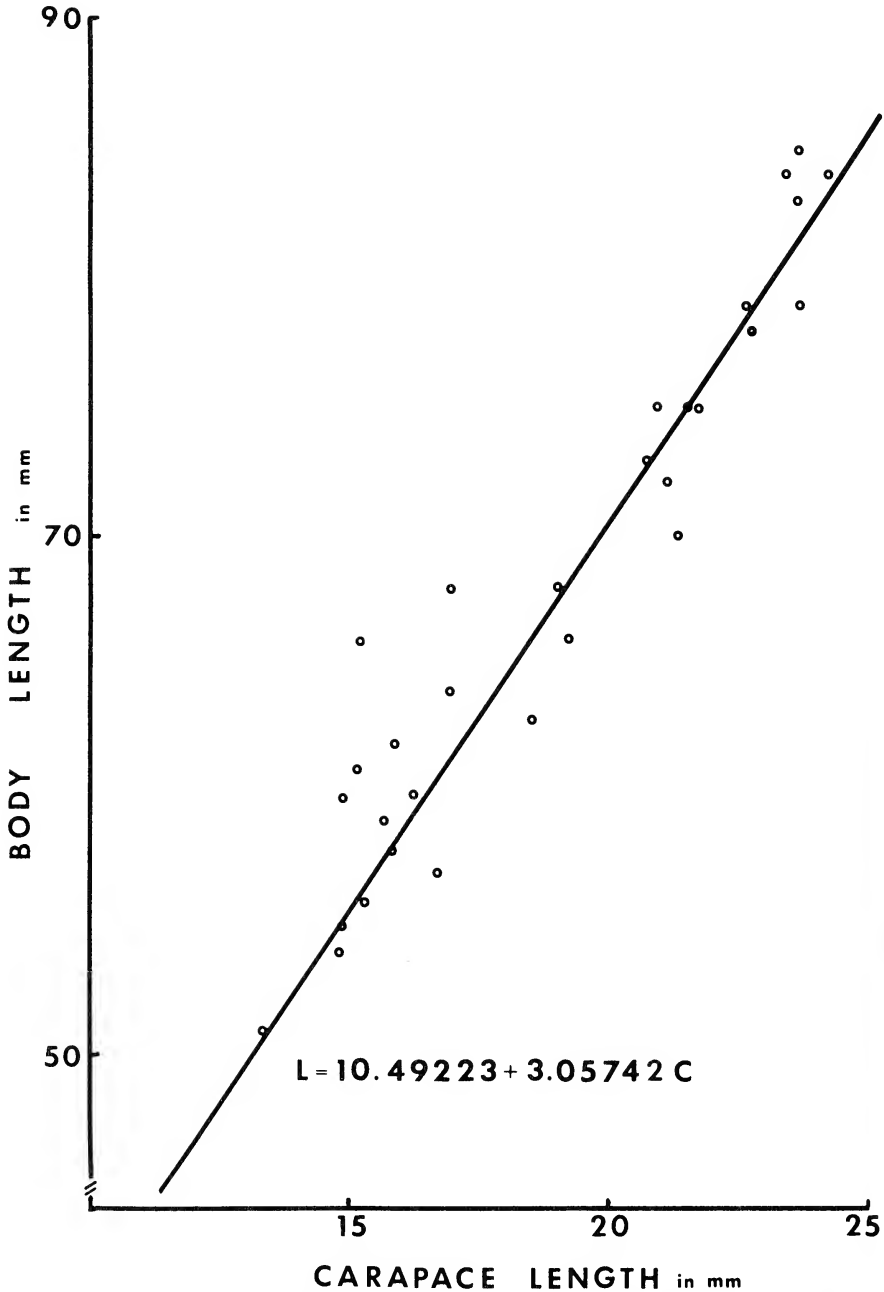


Figure 5. Relationship between carapace length (C) and body length (L) of *M. ohione*.

reported seasonal movement of *M. ohione* with maximum abundances in 5 ppt salinity. Gunter (1937) found *M. ohione* in a salinity range of 1.38 – 14.24 ppt in bay waters. Viosca (1957) noted that *M. ohione* also inhabited freshwater lakes

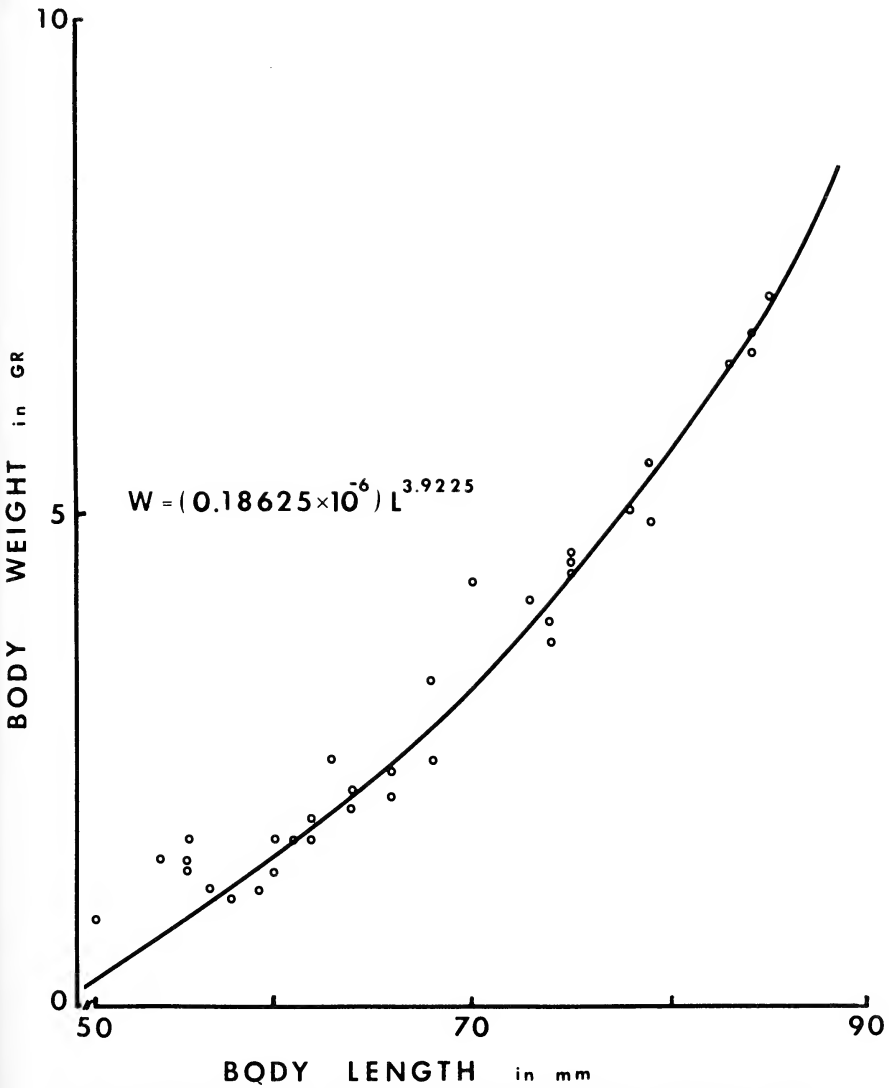


Figure 6. Relationship between body length (L) and body weight (W) of *M. ohione*.

in the flood plains down to the edge of salt water in Louisiana. Dugan (1971, 1972) discussed its salinity tolerances in the laboratory and indicated that gravid females would spawn and the larvae hatch in freshwater. The larvae needed brackish water. Reimer, *et al.*, (1974) have suggested that salinity near 15 ppt acted as a barrier to movement of shrimp into Galveston Bay.

Hughes, *et al.*, (1973) reported that current chamber experiments indicated gravid *M. acanthurus* tend to swim consistently downstream whereas nongravid females tend to swim upstream. This would seem to substantiate the general feeling that the gravid female is stimulated to return to brackish water for the release of

her spawn. This probably occurs in *M. ohione* and accounts for the large number taken in bay systems (Gunter, 1937; Reimer, *et al.,* 1974). It would seem most likely that *M. ohione* spawn and the eggs hatch in the mouth of the rivers or in the upper estuary where the salinity is lower than 15 ppt. The larvae move to the bay area where salinities are higher (between 10 - 20 ppt), for the larvae need more than 15 ppt salinity during larval development periods (Dugan, 1972). After metamorphosis into the post-larval stage, they presumably migrate contranatant to the river where salinities are low (less than 15 ppt). Growth continues in freshwater until the next spawning when they again migrate to the estuaries.

High mortality was associated with high water temperatures occurring during molting of the shrimp. The mortality during molting occurred when late afternoon temperatures reached 31.5 C and higher. Experimental temperatures reached 32 C for several days and non-molting animals were able to survive. None survived at a peak temperature of 32.9 C after molting. In general, temperature and dissolved oxygen would be highly interacted in molting and mortality of crustacean, however, a biological filter system and a MillionAire air stone (Figure 1) presumably provided adequate dissolved oxygen in this experiment. Temperature was considered to be the primary factor affecting molting and mortality.

Reimer, *et al.,* (1974) have suggested that temperature near 30 C represented the upper limit of tolerance of *M. ohione* in the Galveston Bay area. My data showed that 31.5 C was the upper tolerance limit of *M. ohione* in laboratory tanks. It would appear that temperature regulates the distribution of this species by interferring with the actual molting process. Since *Macrobrachium* must go through a prespawning molt, temperature control is critical.

*M. ohione* lives in the intake canal of the P. H. Robinson Generating Station, Bacliff, Texas, during periods of low salinity. Temperatures of intake water during June and July of 1974 ranged from a daily minimum of 24.4 to 30.0 C and a maximum of 26.6 to 32.2 C. These temperatures are similar to those recorded in my tanks (25.7 to 28.7 C in the morning and 28.5 to 32.9 C in the afternoon) and they indicate that *M. ohione* needs to seek cool area to survive during the hot part of the summer.

The relationship between temperature (T) and the % mortality (based on number of shrimp in all 6 tanks after molting) is presented graphically in Figure 7. It shows that temperature is directly correlated with mortality. It would seem that temperatures for juvenile and adult *M. ohione,* in ponds, will need to be under 30 to 31 C.

*Growth*

The relationship between initial ($l_t$) and terminal body length ($L_{t+T}$) was plotted and a Walford line was found $L_{t+T} = 96.79431(1-e^{-2.40192}) + e^{-2.40192}l_t$. Calculated maximum body length was 96.79 mm.

The calculated maximum body length, 96.79 mm, compared favorably with most reports (Table 2) from the literature. It would seem, therefore, under artificial conditions, one might expect the size of adult populations to be similar to natural populations.

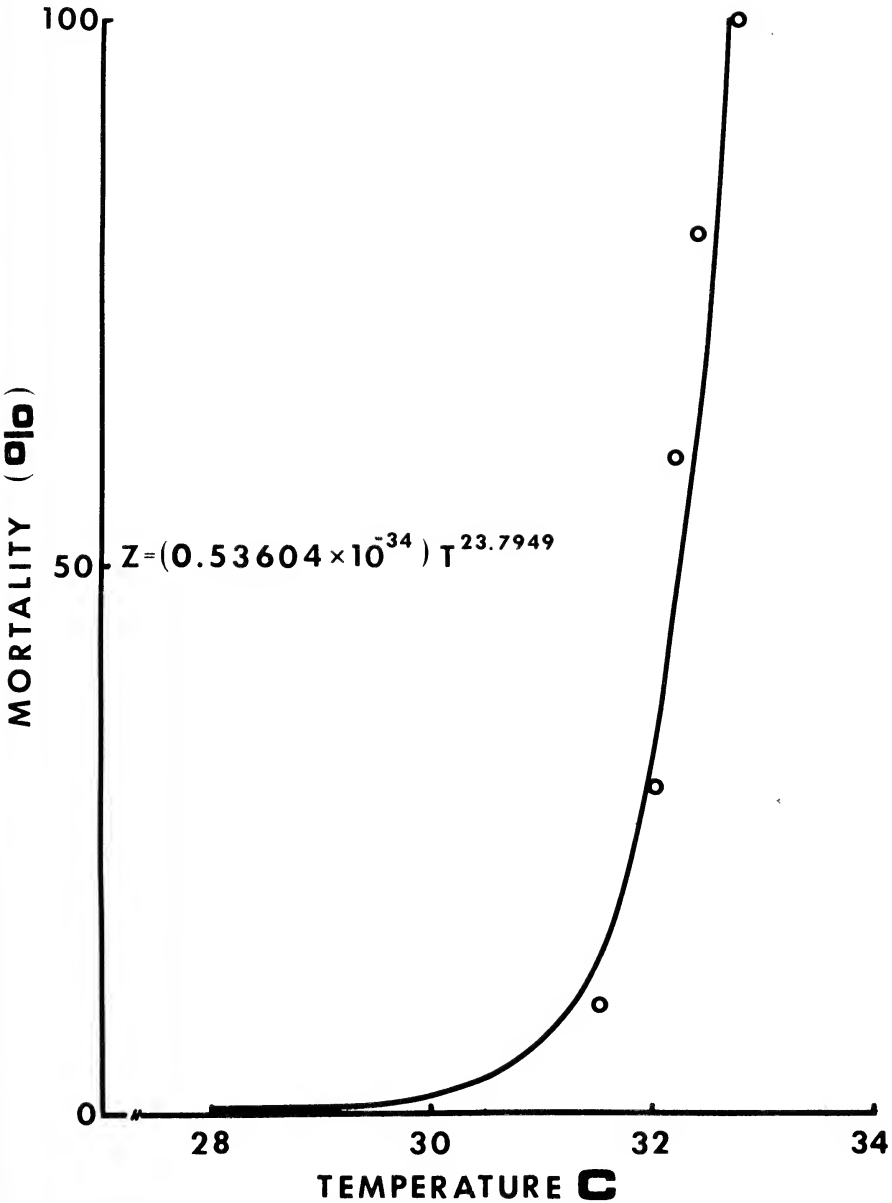


Figure 7. Relationship between temperature (T) and percent mortality (Z) of *M. ohione*.

Relationship between carapace length (C) and body length (L) was plotted and showed a linear equation, L = 10.49223 + 2.05742 C. Calculated maximum carapace length substituting from this formula, was 28.23 mm. The body length (L) and body weight (W) relationship was plotted and formulated with $W = (0.18625 \times 10^{-6})L^{3.9225}$. Calculated maximum body weight was 11.47 g.

TABLE 2

Maximum Lengths of *M. ohione* Reported From the Literature.

| Author | Length | Sex |
|---|---|---|
| Schmitt (1933) | 103 mm | F |
| McCormick (1934) | 96 mm | ? |
| Gunter (1937) | 93 mm | ? |
| Hedgpeth (1959) | 100 mm | F |
| Dugan (1971) | 100 mm | F |
| Reimer, *et al.*, (1974) | 85 mm | F |

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# THE ECOLOGY OF A BRANCHIOBDELLID (ANNELIDA : OLIGOCHAETA) FROM A TEXAS POND

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ABSTRACT

Seasonal changes in population structure of the branchiobdellid *Cambarincola vitrea* inhabiting crayfishes of a Texas farm pond were investigated. Worm population density and reproduction were correlated with fluctuations in the physical habitat, the individual host size class, and the apparent host molt condition. Mean worm load and cocoon deposition reflected several meteorological disturbances of the pond during the period of study (June 1972 to May 1973). Regardless of month of collection, molting hosts showed significantly reduced branchiobdellid populations compared to intermolt crayfishes. A curvi-linear relationship exists between increasing host size and worm load in favor of larger animals, indicating the high molt frequency among younger crayfishes. Cocoon deposition fluctuated in a pattern similar to that of the adult worms. The branchial cavities and pleopods appeared to be preferred sites for cocoon deposition.

## INTRODUCTION

Branchiobdellids are annelid ecto-inhabitants of the gills and exoskeleton of freshwater crustaceans, chiefly crayfishes. Although quite common in freshwater environs of North America, knowledge of these oligochaetes is limited. In particular the extent of interaction between host and annelid remains obscure. While several branchiobdellid species have been reported to be truly parasitic (Holt, 1963), the majority are considered commensals. Young (1966) has demonstrated that a viable host is a necessary condition for branchiobdellid reproduction.

Previous investigations have been primarily limited to taxonomic considerations. Goodnight (1940) revised the family, while reviewing the scattered early literature. Holt (1954, 1955, 1960, 1963) and Hoffman (1963) have added new species descriptions. Although several ecological reports have been published (Berry and Holt, 1959; Penn, 1959; McMannus, 1960; Young, 1966; Bishop, 1968), specific information is lacking concerning branchiobdellid activities within a habitat prone to seasonal meteorological extremes. Such habitats are common to Texas in the form of small farm ponds. The branchiobdellid *Cambarincola vitrea* Ellis is frequently found inhabiting crayfishes of this region, including the pond-dwelling *Procambarus simulans* Faxon.

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It was the purpose of this investigation to study seasonal changes in branchiobdellid population structure within a Texas pond. Branchiobdellid response was assessed on the basis of worm load and reproduction correlated with host factors and fluctuations of the physical environment.

## METHODS AND MATERIALS

The study site was located 3.5 mi north of Denton, Texas, on public land adjacent to Interstate Highway 35. The pond is a man-made depression with a silty black mud substrate. Water depth varied from 2.2 m in the spring to complete dryness in the late summer and early fall.

A prominent member of the aquatic fauna is the crayfish *Procambarus simulans* (family Astacidae, subfamily Cambarinae), which is among the most common of the Texas crayfishes. The only branchiobdellid found within the study site was *Cambarincola vitrea.* Keys prepared by Hoffman (1963) and Goodnight (1940) aided in the identification.

Crayfishes were collected with a hand net and immediately placed into individual bottles containing a saturated solution of chlorobutanol. Monthly collections were carried out (except September when the pond was dry). At each sampling, 50 crayfishes of various size classes were treated for removal of resident branchiobdellids, and the worm load determined. All specimens were subsequently fixed and stored in 70% isopropyl alcohol.

Crayfishes were also individually analyzed for carapace length (in cm) and apparent molt condition. Determination of molt condition was restricted to molt and intermolt categories. The former included postmolt animals. Criteria included: (1) evidence of pronounced imbibition; (2) presence of encrusting algae and/or deposited insect ova on the exoskeleton; and (3) extent of gastrolith development.

Branchiobdellid cocoon deposition and distribution on the host body were also investigated. Accordingly, all animals were first screened for the presence of cocoons. Crayfishes were then grouped into 6 size classes (0-1.9, 2.0-2.9, 3.0-3.9, 4.0-4.9, 5.0-5.9, and 6.0-6.9 cm carapace length), and cocoon deposition measured as a function of this parameter.

Finally, 10 cocoon-bearing hosts from each monthly sample were carefully biopsied under a dissecting microscope to determine the relative distribution of cocoons. Areas examined included branchial chamber, cephalothorax, pereiopods, abdominal segments, and pleopods. Relative percentages were calculated for each area.

## RESULTS

Mean worm load per host (Figure 1) decreased significantly when the pond dried up in September and during a freeze in early January. Infestation varied from a high of 98.8 worms/host in August to 16.6 worms/host in March. Gradual recovery was noted when sampling was terminated in June.

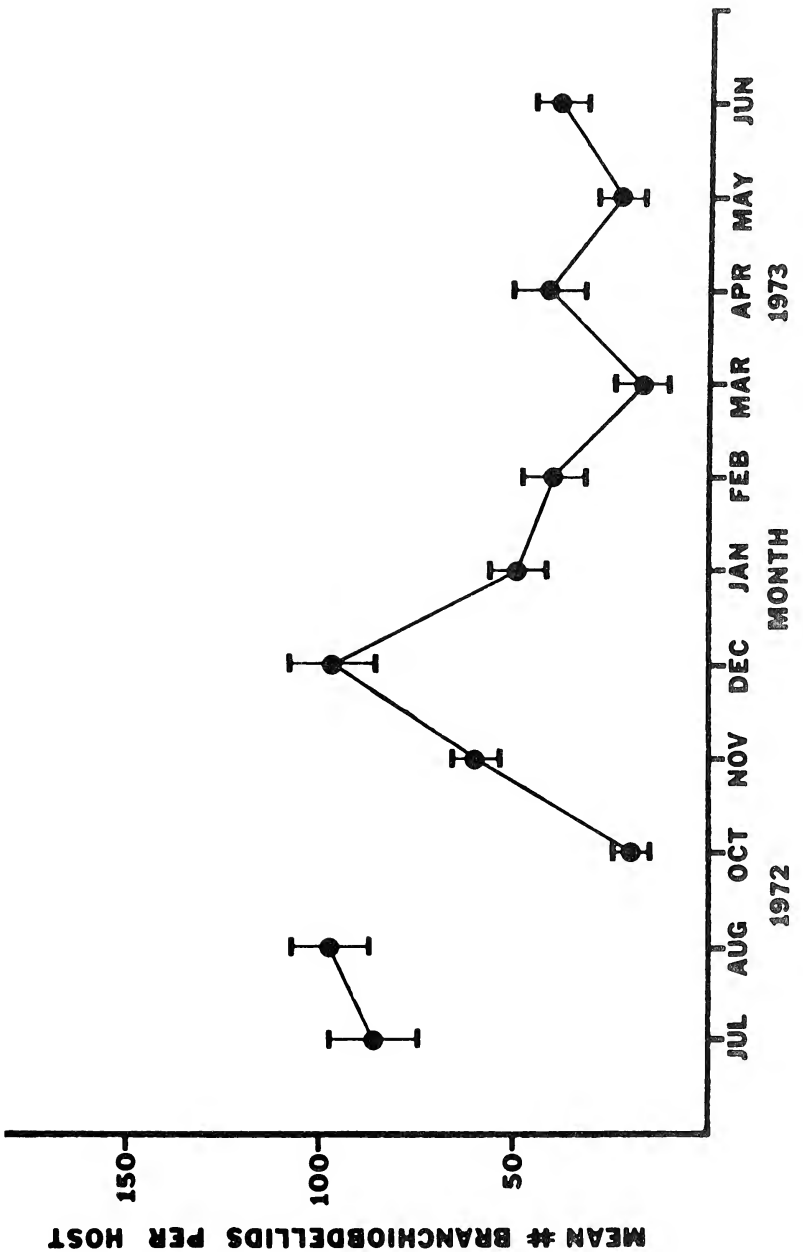


Figure 1. Monthly fluctuation in branchiobdellid number/given host. Each mean (±1 Standard Deviation) represents a sample size of 50 crayfishes.

Observed differences in worm number between successive samples suggested that host factors were also involved. When mean worm load was tested against

host molt status (Figure 2), intermolt crayfishes were observed to maintain larger branchiobdellid populations than the corresponding molted animals. Greater variation in worm number was also noted for intermolt hosts. When mean worm load was plotted against host size (Figure 3), a curvi-linear relationship was obtained in favor of larger crayfishes.

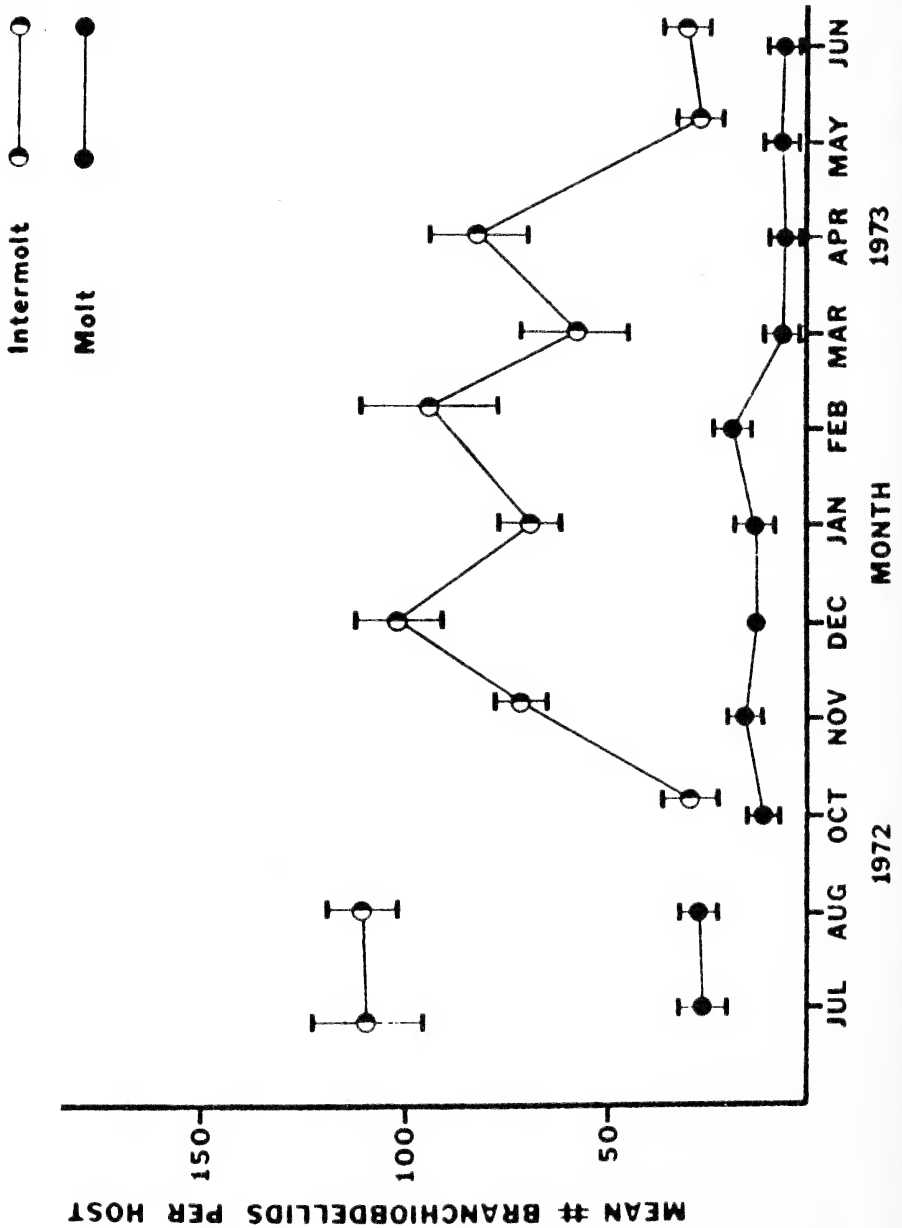


Figure 2. The relationship of branchiobdellid number to host molt condition. Each mean (±1 Standard Deviation) represents a variable number of crayfishes.

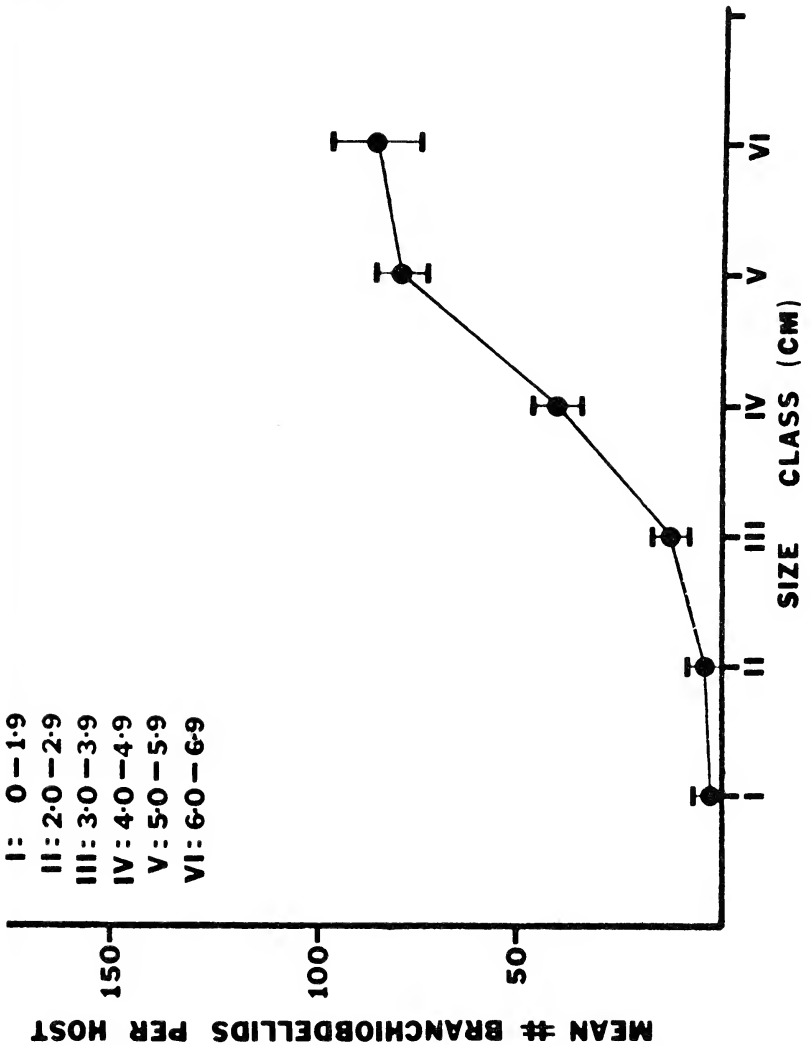


Figure 3. The relationship of branchiobdellid number to host size. Each mean (±1 Standard Deviation) represents a variable number of crayfishes.

As with the adult worms, cocoon deposition (Figure 4) reflected seasonal changes in the abiotic habitat. In August, nearly 90% of all crayfishes sampled were found to bear cocoons. The subsequent October sample showed a decline to 63%. A second and much sharper decrease (to 22%) in cocoon-bearing animals was observed in March, with subsequent recovery indicated in June.

Cocoon deposition was further assessed as a function of host size (Table 1). Invariably, 1st-year crayfishes (0-1.9 cm) were devoid of cocoons. Only isolated

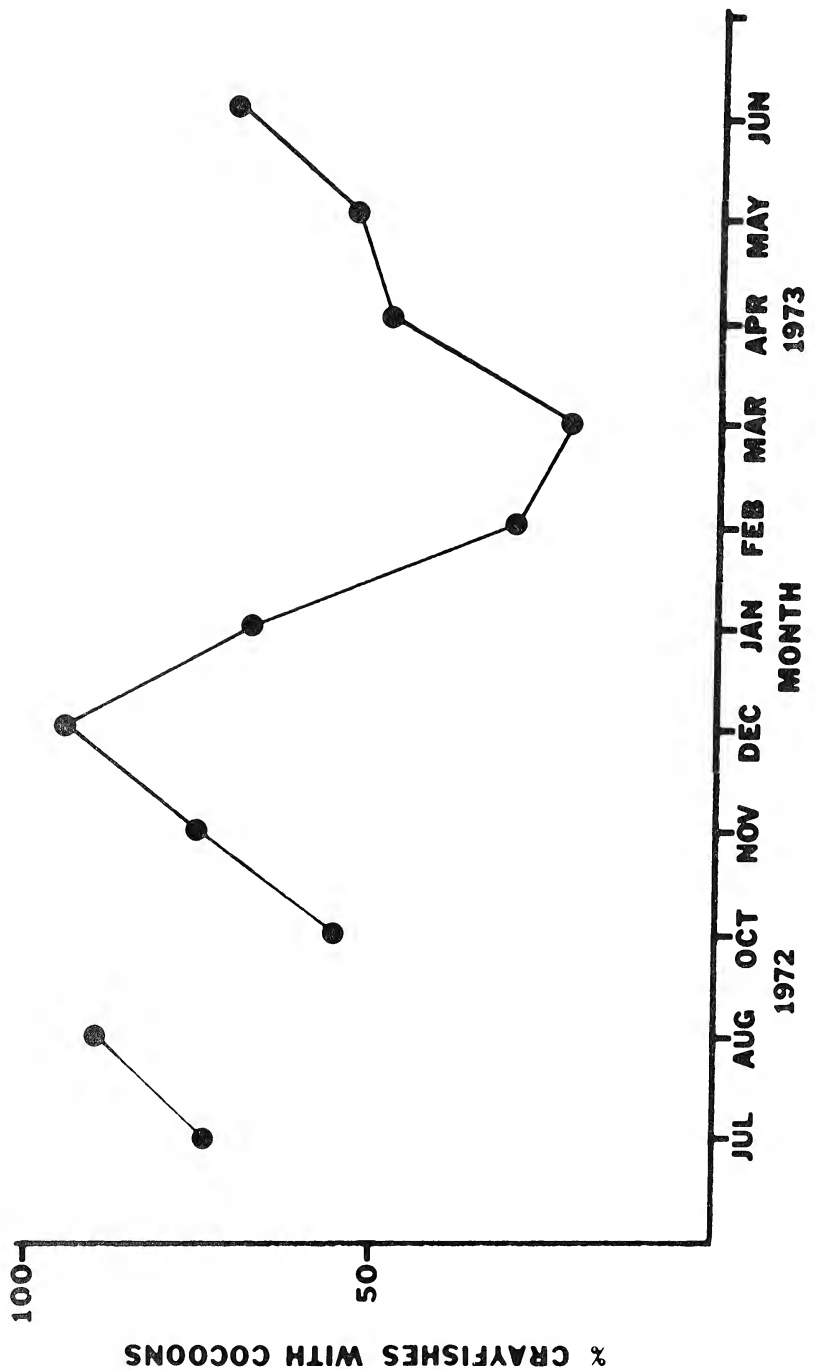


Figure 4. Percent occurrence of branchiobdellid cocoons on crayfishes from monthly collections.

TABLE 1

Monthly Cocoon Occurrence According to Host Size.

| Size Range (cm) | Percent crayfishes exhibiting cocoons | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | JUN. | JUL. | AUG. | OCT. | NOV. | DEC. | JAN. | FEB. | MAR. | APR. | MAY | TOTAL |
| 0 – 1.9 | * | 0 | * | * | 0 | 0 | 0 | * | 0.0 | 0 | * | 0.0 |
| 2.0 – 2.9 | 0 | 0 | 0 | * | 0 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| 3.0 – 3.9 | 72 | 80 | 33 | 24 | * | 100 | 100 | 0 | 8.3 | 27 | 50 | 49.4 |
| 4.0 – 4.9 | 82 | 66 | 85 | 28 | 70 | 100 | 60 | 0 | 0.0 | 42 | 85 | 56.0 |
| 5.0 – 5.9 | 66 | 83 | 100 | 66 | 94 | 100 | 43 | 0 | 100.0 | * | 100 | 75.2 |
| 6.0 – 6.9 | * | 86 | 100 | 66 | 100 | 100 | 75 | 74 | 62.0 | 77 | * | 82.2 |

* Not obtained in monthly sample.

hosts in the 2.0-2.9 cm range possessed cocoons. Medium-sized crayfishes (3.0-4.9 cm) appeared to vary as suitable choices for deposition, while larger hosts (5.0-6.9 cm) maintained a high % throughout the year.

Relative distribution of branchiobdellid cocoons on the host body is presented in Table 2 (since each monthly sample was limited to 10 crayfishes, results are grouped to coincide with the 4 seasons). The branchial chambers and pleopods were employed as primary deposition sites throughout the year. The first abdominal

TABLE 2

Relative Distribution of Cocoons on Crayfishes by Seasons.

| Body Region | Percent of total deposited cocoons | | | | |
|---|---|---|---|---|---|
| | SUMMER | FALL | WINTER | SPRING | OVERALL |
| Cephalothorax | 0.0 | 0.0 | 5.9 | 0.0 | 1.5 |
| 1st Abdominal seg. | 2.3 | 1.3 | 6.8 | 11.0 | 5.3 |
| 2nd Abdominal seg. | 1.6 | 16.4 | 13.8 | 13.3 | 11.3 |
| 34d Abdominal seg. | 0.3 | 12.1 | 5.3 | 1.8 | 4.8 |
| 4th Abdominal seg. | 0.4 | 1.9 | 1.9 | 1.8 | 1.5 |
| 5th Abdominal seg. | 0.3 | 0.0 | 0.1 | 0.1 | 0.2 |
| 6th Abdominal seg. | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7th Abdominal seg. | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Pleopods | 13.5 | 16.6 | 31.6 | 25.2 | 21.7 |
| Branchial cavity | 81.5 | 50.9 | 28.6 | 46.9 | 52.0 |
| Pereiopods | 0.3 | 0.9 | 6.0 | 0.0 | 1.8 |

segments ranked as secondary sites. Pereiopods, terminal abdominal segments, and cephalothorax generally were less favored for deposition.

## DISCUSSION

Major decreases in worm load and reproduction of *Cambarincola vitrea* reflect meteorological disturbances of the pond in September and early January. Seasonal reductions in branchiobdellid population density have been reported previously (Young, 1966; Bishop, 1968), with selective mortality and diminished reproduction cited as possible mechanisms. It is likely that such severe disturbances of the habitat medium accelerate normal selective processes (e.g., juvenile mortality, decreased reproductive rates) that govern the observed fall and spring reductions in branchiobdellid population density.

In addition to abiotic factors, the results indicate a preferential host selection by *Cambarincola vitrea* in favor of larger intermolt crayfishes. Replacement of the host exoskeleton has been shown to cause irreversible cessation of cocoon development (Young, 1966) and the mass evacuation of the affected host by adult worms (Koepp, 1975). Accordingly, the lengthy intermolt period of larger crayfishes makes them ideal for long-term branchiobdellid colonization. The increased body size also provides a greater surface area for worm inhabitation, cocoon deposition, and availability of food.

Contrary to previous reports relative to the genus *Cambarincola* (McManus, 1960; Young, 1966; Bishop, 1968), the branchial cavities and pleopods appear to be the principal sites for cocoon deposition. While not specifically investigated, the use of these ventilative loci may reflect an adaptation to satisfy the respiratory needs of the developing embryos.

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# LIMNOLOGICAL THESES AND DISSERTATIONS CONCERNING TEXAS WATERS, 1897–1976

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ABSTRACT

Seven hundred seventy-seven titles of limnologically-oriented theses and dissertations were collected over a five-year period by the author. Universities and colleges in Texas and bordering states were surveyed by various methods. The titles are indexed according to key words in the respective titles. Although the major period of time covered is from 1897 through 1975, there is a 1976 ADDENDUM which includes those listings currently in progress.

## INTRODUCTION

In order to provide easier access to information on subjects related to limnology in Texas, this compilation has been made of theses and dissertations in that area. This report is the result of five years of continuous literature surveys conducted among the various educational institutions in Texas and contiguous states. The survey methods included a letter addressed to the research section of each institutional library, letters to the departmental chairmen of all fields related to limnology, contacts by telephone, and personal visits. Additional listings were obtained from commencement programs of various graduation proceedings. A base for the work was provided by two publications:

Clark, W.J., 1966–Publications, personnel, and government organizations related to the limnology, aquatic biology, ichthyology of the inland waters of Texas. Technical Report No. 5, Water Resources Institute. Texas A&M University. 102 pp.

Ferguson, S., 1970–A bibliography of the water-related theses and dissertations written at the University of Texas at Austin 1897-1970. Report No. 68, Center for Research in Water Resources, University of Texas at Austin.

The listing of theses and dissertations is grouped by college or university. To facilitate use of the list, however, the entries have been numbered and are indexed below (SUBJECT INDEX). In most cases this index is based upon key

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words contained in the titles, as it has not been possible for the author to review each publication.

It should be noted that copies of the theses and dissertations compiled herein are available from the library of the institution where the work was completed. Correspondence suggesting additions, corrections, and deletions to this report is welcome, and should be addressed to the author: John Pettitt, Route 2, Box 328 P, Midlothian, TX 76065.

ANGELO STATE UNIVERSITY

1. McClung, G. Dan, 1975–The phylogenetic relationships of some species of *Gambusia* occurring in Texas. M.S.

BAYLOR UNIVERSITY

2. Alexander, T.C., 1940–A biological survey of the Bosque River system, Waco, Texas. M.S.
3. Bane, Carol, 1975–The limnology of aquatic systems of Big Bend National Park with emphasis on water quality. M.S. (In progress)
4. Bothner, G.L., 1957–An age and growth study of *Ictiobus bubalus* in Lake Waco, Texas. M.S.
5. Chen, T., 1969–Age and growth of the white crappie, *Poxomis annularis* (Rafinesque) in Lake Waco, Texas. M.S.
6. Davidson, F.F.,1933–The algae of McLennan County, Waco, Texas. M.S.
7. Harrell, H.L., 1975–Fishes of Devil's River. M.S.
8. Kennedy, S., 1975–The ecology of the lean springs pupfish, *Cyprinodon bovinus*. M.S. (In progress)
9. Lade, O.R., 1934–Food of fish in McLennan County. M.A.
10. Lewand, R.L., 1967–The geomorphic evolution of the Leon River system. M.S.
11. Lukins, David L., 1975–Production and diversity of aufwuchs populations in thermal effluents. M.S. (In progress)
12. Moore, T.H., 1968–Geochemistry of waters of Hog Creek Basin; Hamilton, Coryell and Bosque Counties, Texas. M.S.
13. Moore, T.D., 1965–Growth and development of the Brazos River Authority. M.S.
14. Proctor, C.V., 1967–The North Bosque watershed: Inventory of a drainage basin. M.S.
15. Stewart, M.B.W., 1967–An ecological study of the bacterial flora of Comanche Spring and Harris Creek. M.S.
16. Scott, C., 1975–Factors influencing transverse stability in fishes. M.S.
17. Venables, B.J., 1972–Some effects of thermal history on the large mouth black bass, *Micropterus salmoides* (Lacepede) of two central Texas reservoirs receiving power plant effluents. M.S.

18. Waldorf, G.W., 1942–Fall and winter food habits of fish of the Bosque River system, Waco, Texas. M.A.

19. West, G.H., 1932–Fishes of McLennan County. M.A.

20. Wilson, K.R., 1975–Phosphate uptake kinetics of natural phytoplankton populations as affected by temperature. M.S. (In progress)

21. Wysong, M., 1973–The ostracods *Cypridopsis vidua*: Physiological response due to thermal stress. M.S.

EAST TEXAS STATE UNIVERSITY

22. Bradley, M.J., 1975–Species diversity of fish populations in three Northeast Texas rivers. M.S.

23. Campbell, D., 1975–A study of feeding habits and condition of *Micropterus salmoides* in Lake Davy Crockett, Texas. M.S.

24. Clay, David, 1975–An age-growth study of black bass in Lake Davy Crockett, Texas. M.S.

25. Duncan, C.W., 1971–Species diversity of benthic macroinvertebrates in the South Sulphur River. M.S.

26. Glueck, D., 1975–A vegetative analysis of beaver habitats along the South Sulphur River. M.S.

27. Henry, D., 1975–Food competition between channel catfish and black bullheads in newly constructed farm ponds. M.S.

28. Long, H., 1963–A study of the algae population of the Hefley-Taylor Lake of Commerce, Texas. M.S.

29. Patterson, B., 1975–Population analysis of red-eared turtles in an East Texas farm pond. M.S.

30. Roberts, D., 1975–An ecological study of red-eared turtles in an East Texas farm pond. M.S.

31. Schultz, G.G., 1968–Chlorinated hydrocarbon residues in aquatic plants. M.S.

32. Seglem, M., 1975–Effects of Toxic metals from industrial plating solutions on large mouth bass, *Micropterus salmoides*. M.S.

LAMAR UNIVERSITY

33. Ashcraft, J.A., 1973–Physio-chemical conditions and macrobenthic community structure in the lower Neches River. M.S.

34. Chaiyarach, S., 1974–Acute toxicity of toxaphene and ordram to four species of aquatic organisms. M.S.

35. Duplechin, J., 1975–The effects of municipal sewage effluent, municipal surface runoff, and rice fields irrigation water on community structure of macrobenthos in Hillebrandt Bayou. M.S.

36. Gibson, G.E., 1974–Seasonal variations in physico-chemical conditions of sewage effluent through the Beaumont, Texas, treatment plant and receiving stream. M.S.

37. Howard, R., 1973–Physico-chemical conditions and communities of macrobenthos in the lower Neches River.

38. Lewis, S.P., 1974–Physico-chemical conditions and macrobenthos of Village Creek. M.S.

39. Marsh, C.E., Jr., 1973–Seasonal abundance and community structure of algae and rotifers in Massey Lake, Southeast Texas. M.S.

40. Olson, K.R., 1971–Salinity effects on the TLm for mercury, copper, and chromium for *Rangia cuneata*. M.S.

41. Patterson, L.D., 1971–Physico-chemical conditions and community structure of benthic macroinvertebrates in the Neches River in the vicinity of the salt water barrier. M.S.

42. Ratananun, V., 1974–Toxicity of the insecticide Sevin and the herbicide Propanil to four aquatic species. M.S.

43. Sala, G.M., 1975–Benthic macroinvertebrates in the Beaumont tertiary sewage treatment ponds and receiving stream. M.S.

44. Siegal, B.A., 1975–Physico-chemical conditions and community metabolism in a Southeast Texas oil refinery holding pond. M.S.

45. Welch, M.O., 1973–Seasonal variation of physico-chemical characteristics and benthic macroinvertebrates in a southeast Texas meander scar lake. M.S.

MIDWESTERN UNIVERSITY

46. Bustetter, L.A., 1972–Membrane filter characteristics of fecal streptococcal pollution in Holliday Creek, Wichita County, Texas. M.S.

47. Whitt, J.W., 1971–An ecological study of Cladoceran populations and selected littoral regions of Lake Wichita. M.S.

NORTH TEXAS STATE UNIVERSITY

48. Abshire, Robert Louis, 1970–Immunoflorescence as a method for the rapid identification of *Streptococcus foecalis* in water. Ph.D.

49. Alam, I.M., 1976–Phytohormone effects in algae. Ph.D.(In progress)

50. Allison, Richard C., 1966–Microbiotic cycles in Lake Hefner. M.S.

51. Bagwell, Russel L., 1938–A study of the phytoplankton population of Lake Dallas. M.S.

52. Beddow, D.G., 1966–Some physiological effects of chlorine upon two chlorine resistant algae. M.S.

53. Benson, D., 1976–Production and energy metabolism in Midge and Mayfly populations in North Central Texas pond ecosystem. M.S. (In progress)

54. Boswell, James T., 1976–The use of adenosine triphosphate (ATP) assays in describing the Limnology of Moss Reservoir. Ph.D. (In progress)

55. Brooks, Benjy F., 1940–A bio-chemical comparative study of the plankton in Lake Dallas and Pecan Creek. M.S.

56. Brown, A.V., 1974–Ecological energetics of the Dobsonfly, *Corydalus cornutus*. Ph.D.

57. Brown, J.H., 1957–An investigation of methods for the concentration of chemical compounds produced by actinomycetes and their relation to tastes and odors in municipal water supplies. M.S.

58. Cahoon, R., 1976–Decomposition rates in a small North Central Texas pond ecosystem. M.S. (In progress)

59. Chapman, R., 1973–Effects of ethephon on *Scenedesmus quadricauda*. M.S.

60. Cloud, Thomas J., 1973–Drift of aquatic insects in the Brazos River, Texas. M.S.

61. Cooper, W.A., 1950–Age, growth, and feeding habits of the large-mouthed black bass (*Micropterus salmoides*) and the spotted bass (*Micropterus punctulatus* sp.) in North-Central and East Texas lakes. M.S.

62. Cook, V., 1946–A quantitative and qualitative bacterial analysis of Pecan Creek. M.S.

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64. Durrett, Charles Wade, 1973–Density, distribution, production, and drift of benthic fauna in a reservoir receiving thermal discharges from a steam-electric generating plant. M.S.

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67. Elliott, J.M., 1948–The age and rate of growth of the black crappie, *Pomoxis nigromaculatus* (Le Seur), and the white crappie, *Pomoxis annularis* (Rafinesque), in the Koon Kreek Klub Lakes, Texas. M.S.

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71. Fair, Helena Juengerom Ann., 1952–Toxicity studies of aquatic actinomycetes. M.S.

72. Farrell, R., 1975–Sporulation control in heterocystous blue-green algae. M.S. (In progress)

73. Foster, R.M., 1937–A yearly study of the bacterial flora of Lake Dallas water with special reference to sanitation. M.S.

74. Foerster, J.W., 1964–The synecology of phyco-periphyton in oligotrophic lakes. M.S.

75. Friday, Gary Phillip, 1971–Spring food habits of *Corydalus cornutus* in the Brazos River, Texas. M.S.

76. Goodman, J.W., 1959–A water quality study of Lake Texoma. M.S.

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95. Kelly, M.H., 1975–Primary productivity and community metabolism in a small North Central Texas pond ecosystem. M.S.

96. Koen, L.D., 1972–The food habits of bluegills in a lake receiving heated effluent. M.S.

97. Lamb, L.D., 1938–A comparative study of the bottom fauna of four Texas lakes. M.S.

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99. Legett, J.H., 1940–A net plankton survey of a small perennial pond. M.S.

100. Maki, Alan Walter, 1971–Influence of sublethal pesticide levels on respiratory activity of selected aquatic animals. M.S.

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104. McCormick, W.C., 1954–The cultural, physiological, morphological, and chemical characteristics of an actinomycete from Lake Waco. M.S.

105. McDaniel, M.D., 1973–Trophogenic ecology of selected Southwestern reservoirs. Ph.D.

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111. Mitterer, G., 1976–Distribution, abundance and food habits of larval fishes in a reservoir receiving heated effluents from a power plant. M.S. (In progress)

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113. Newton, C.E., 1973–Carbon flux in reservoir sediments. Ph.D.

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115. Parsons, W.M., 1966–Dispersal of algae and protozoa by selected Odonata. M.S.

116. Peterson, J.A., 1972–Indole-3-acetic acid effects on the nucleic acids of *Chlorella pyrenoidosa*. M.S.

117. Pipes, W.O., 1955–An investigation of naturally occurring tastes and odors from freshwater. M.S.

118. Redden, D.R., 1949–The physical, chemical and biological factors affecting algae blooms in freshwater reservoirs. M.S.

119. Revill, D.L., 1966–Transport of viable dissemules of algae and protozoa by selected diptera. M.S.

120. Rhame, R.E., 1973–Life cycle and ecology of the Hydropsychidae (Trichoptera) of the Brazos River, Texas. Ph.D.

121. Richey, Harvey, 1973–Studies of selected Cyanophyta response to varying geosmin concentrations. M.S.

122. Russell, J.C., 1952–Biological indices of stream pollution. M.S.

123. Sams, Barry L., 1976–Comparative chemistry of thermally stressed North Lake and its water source, Elm Fork of the Trinity River. M.S.

124. Sherman, R.C., 1946–Seasonal food habits of five species of Texas Centrarchids. M.S.

125. Sivels, H.C., 1945–A preliminary investigation of the effects of various fertilizers on plankton and fish production in small Texas ponds. M.S.

126. Smith, J.A., 1973–Primary productiivity and nutrient relationships in Garza-Little Elm Reservoir. Ph.D.

127. Smith, C.G., 1948–The reproductive cycles of five species of Texas Centrarchids. M.S.

128. Smith, B.A., 1938–A physical and chemical investigation of Eagle Mountain Lake with reference to biological productivity. M.S.

129. Smith, G.A., 1976–Population dynamics and energy metabolism of microcrustacean zooplankters in a North Central Texas pond ecosystem. M.S.

130. Solon, B.M., 1970–Passive dispersal of algae and protozoa internally and externally by selected aquatic insects. Ph.D.

131. Stark, W.P., 1972–The stoneflies (Plecoptera) of Oklahoma. M.S.

132. Stiles, J.C., 1968–A bacteriological survey of a freshwater reservoir. M.S.

133. Stuart, T.J., 1975–Effects of entrainment and passage through an electric power generator condenser and thermal effluent on phytoplankton populations and their photosynthetic activity. M.S. (In progress)

134. Szczytko, Stanley W., 1978–The biology and systematics of the genus *Isoperla* (Plecoptera) of western North America. Ph.D. (In progress)

135. Trotter, D.M., 1969–A comparison of the carbon dioxide to oxygen rate of change methods for measuring primary productivity. M.S.

136. Tsai, C.E.J., 1973–Methylglyocal metabolism in *Scenedesmus quadricauda*. M.S.

137. Vaught, G.L., 1972 The life history and ecology of the stone fly *Neoperla clyment* (Newman). M.S.

138. Venables, B., 1976–Thermal ecology of large-mouth bass in North Central Texas. Ph.D.

139. Welch, H., 1939–A chemical investigation of Lake Dallas to determine the factors influencing plankton growth. M.S.

140. Wilcox, D.P., 1976–The relationship between biomass and primary production in the phytoplankton community of a mesotrophic Texas reservoir. M.S. (In progress)

141. Woods, A.M., 1937–A comparative investigation of the water of Lake Bridgeport with reference to plant and animal life. M.S.

142. Wyatt, J.T., 1969–Selected physiological and bio-chemical studies on blue-green algae. Ph.D.

OKLAHOMA STATE UNIVERSITY

143. Whiteside, Bobby Gene, 1964–The biology of the white crappie *Poxomis annularis*, in Lake Texoma, Oklahoma. M.S.

PAN AMERICAN UNIVERSITY

144. Penn, G.J., 1974–Seasonal periodicity of dominant, benthic, marine macroalgae of the north jetty, Brazos-Santiago Pass, Texas, as related to environmental factors. M.S.

RICE UNIVERSITY

145. Crowley, P.H., 1972–Filtering rate inhibition of *Daphnia pulex* in Wintergreen Lake water. M.S.

146. Keegan, G.J., 1970–Water quality management. Optima allocation of pollutant discharges constrained by quality and equity considerations. M.S.

147. McRitchie, R.G., 1968–Effects of thermal and osmotic acclimation on an estuarine gastropod. Ph.D.

SAM HOUSTON STATE UNIVERSITY

148. Altaras, R.M., 1972–The effects of domestic sewage on the distribution of the fish fauna of Harmon Creek, Walker County, Texas. M.A.

149. Barrett, S.R., 1974–A morphological and meristic study of *Menidia* (Bonaparte) populations in the Trinity River system, East Texas (Pisces, Atherinidae). M.A.

150. Boyd, J.F., 1968–A taxonomic and ecological survey of the fish fauna of Bedias Creek, Texas. M.A.

151. Cline, E.M., 1949–A study of the pH variations in the Sam Houston Park pond water. M.A.

152. Harper, A.L., 1969–Salinity tolerances of five species of East Texas teleosts. M.A.

153. King, J.M., 1968–The investigation of freshwater algae populations in Walker and Montgomery counties. M.A.

154. Lasswell, J., 1968–A taxonomic and ecological survey of the fish fauna of the West Fork of the San Jacinto River, Texas. M.S.

155. Thurston, N.I., 1969–An ecological survey of a pondside region in Walker County, Texas. M.A.

156. Tipton, J.L., 1974–The removal of selected elements from a germination medium by *Anabaena* akinetes. M.A.

157. Twidwell, S.R., 1971–Effects of condenser cooling water on community structure of net phytoplankton and related physicochemical features of Lewis Creek Reservoir, Texas. M.A.

158. Wiley, E.O., III, 1972–Geographic variation and systematics of the *Fundulus nottii* (Agassiz) species complex (Pisces, Cyprinodontidae). M.A.

SOUTHERN METHODIST UNIVERSITY

159. Allen, M.R., 1964–An ecological survey of the recent land and freshwater gastropods of Wichita County, Texas. M.S.

160. Anderson, D.S., 1968–The aquatic Coleoptera in the vicinity of Oak Ridge, Tennessee. M.S.

161. Callahan, W.P., 1953–The distribution of coliform bacteria in a reservoir lake as related to dissolved oxygen. M.S.

162. Dean, P.N., 1967–An ecological survey of the living terrestrial and freshwater gastropods of El Paso County, Texas. M.S.

163. Dust, J.V., 1963–A quantitative comparison of chlorophyll "a" with algae count and classification in sewage treatment oxidation ponds. M.S.

164. Erickson, S.J., 1952–Effects of weather conditions on the Turtle Creek plankters near Dallas, Texas. M.S.

165. Ferguson, A.K., 1937–Systematic and ecological notes on the Odonata of the vicinity of Dallas, Texas. M.S.

166. Fullwood, P.D., 1950–A survey of parasitic copepods infesting fish in Dallas County, Texas. M.S.

167. Fullington, R.W., 1969–A study of the distribution, ecology, and taxonomic status in the gastropod families Physidae and Lymnaeidae in Texas. M.S.

168. Flook, J.M., 1970–A survey of Metazoan parasites in Unionid bivalves of Garza-Little Elm Reservoir, Denton County, Texas, with descriptions of a near species of *Rhopalocercous* cercaria.

169. Glass, V., 1949–Report on the aquatic Coleoptera of the Dallas area. M.S.

170. Goff, J.D., 1958–A study of the water quality of the Trinity River in Dallas County. M.S.

171. Hall, C.C., 1950–The Trichoptera of Dallas County, Texas. M.S.

172. Haynes, F., 1942–A study of the food habits of large-mouth black bass fingerlings (*Huro salmoides*) in rearing ponds. M.S.

173. Hasty, M.A., 1950–Plankton studies on Denton Creek, Texas. M.S.

174. Logsdon, W.T., 1967–An ecological survey of the recent land and freshwater gastropods of Kaufman County, Texas. M.S.

175. Majors, E.C., 1964–A partial survey of the freshwater and land gastropods in the area of Harlingen, Cameron County, Texas. M.S.

176. McCleskey, O., 1949–The bionomics of the Culicinae of the Dallas, Texas, area. M.S.

177. Millspaugh, D.D., 1936–Bionomics of the aquatic and semi-aquatic Hemiptera of Dallas County, Texas. M.S.

178. Moore, L.E., 1950–Distribution of mayfly nymphs (Ephemeroptera) in a stream of Dallas County, Texas. M.S.

179. Oliver, K.H., 1952–The effect of herbicides on selected zooplankton. M.S.

180. Patterson, M., 1942–A study on fluctuations in plankton populations in White Rock Lake in winter and spring. M.S.

181. Read, L.B., 1953–The Pelecypoda of Dallas County. M.S.

182. Robbins, D.M., 1968–A seasonal study of certain zooplankton in Turtle Creek, near Dallas, Texas. M.S.

183. Smith, K.K.L., 1972–A limnological survey of Lake Granbury (DeCordova Bend), Texas. M.S.

184. Zischkale, M.A., 1951–The effect of rotenone and some common herbicides on fish food organisms. M.S.

SOUTHWEST TEXAS STATE UNIVERSITY

185. Arnold, C.R., 1962–Oxygen consumption as a factor in the distribution of certain Trichoptera larvae. M.A.

186. Anderson, B.T., 1968–A comparative primary productivity study of three ponds bearing different primary producers. M.A.

187. Barra, T.C., 1976–The effects of a heated effluent on the macroinvertebrate community in Calaveras Lake, Texas. M.S.

188. Barker, E., 1969–A comparative study of the Hydracarina (Acarina) of three permanent ponds. M.A.

189. Bayer, C., 1975–The distribution of Odonates of the Guadalupe River Basin, Texas. M.A.

190. Becker, P.R., 1969–The systematics and seasonal distribution of the Cladocera of Hays County, M.A.

191. Broz, L., 1974—The influence of a deep-storage reservoir and an underground reservoir on the physico-chemical limnology of a Central Texas River. M.A.

192. Bruchmiller, J.P., 1973—Description and keys to the macrophytes of Spring Lake (excluding algae). M.S.

193. Carraway, Joe, 1975—The ecological distribution of water mites of the genus *Arrenurus* in Hays County, Texas. M.A.

194. Chandler, T., 1976—Influence of rain runoff and sewer effluents on the Blanco and San Marcos Rivers. M.S.

195. Colbert, B.K., 1973—Plankton periodicity in two man-made ponds in Central Texas. M.S.

196. Conrads, C., 1972—The effects of selected environmental parameters on the hatching of *Eulimnodia texana*. M.A.

197. Conrads, L.M., 1969—Demography and ecology of the Fern Bank salamander, *Eurycea pterophila*. M.A.

198. Cover, L., 1975—The drift ecology of the Trichoptera of the San Marcos River. M.A.

199. Davis, J., 1975—Ecology of the helminths of *Gambusia affinis* in the area of San Marcos, Texas. M.S.

200. Devall, L.L., 1940—A comparative study of plant dominance in a spring-fed lake. M.S.

201. Dowden, D.L., 1968—Population dynamics of the San Marcos salamander, *Eurycea nana*. M.A.

202. Hamilton, A., 1973—Some taxonomic aspects of certain paedogenetic *Eurycea* of the Blanco River drainage system in Hays and Blanco Counties, Texas. M.A.

203. Henderson, E., 1972—Population dynamics of the *Lepomis cyanellus* (male) X *Lepomis microlophus* (female) hybrid as compared with its reciprocal cross and parental species. M.A.

204. Kent, David, 1971—The effects of a deep-storage reservoir on the benthic macroinvertebrate community of the Guadalupe River, Texas. M.A.

205. Kneupper, A.C., 1971—An investigation of specific taxonomic feature used in the description of *Loptestheria complexiманus*. M.A.

206. Kolb, J., 1975—Age and growth of large-mouth bass in Canyon Lake, Texas. M.S.

207. Litchfield, K., 1975—The macrophytes of the upper San Marcos River. M.A.

208. Lockett, C., 1975—On the classification of 18 Central Texas reservoirs. M.A. (In progress)

209. Martin, M., 1975—A survey of the helminths of the shiners *Notropis venustus* and *Notropis lutrensis* in the area of San Marcos, Texas. M.A.

210. Mayhew, J.J., 1970—Nitrogen and phosphorus dynamics in a 153-kilometer stretch of the Guadalupe River, Texas. M.A.

211. McNatt, R., 1968—Fish species diversity in relation to stream order and physicochemical conditions in the Plum Creek drainage basin. M.A.

212. Munson, J.W., 1946–The distribution of plankton in the San Marcos River, San Marcos, Texas. M.S.

213. Parsons, D., 1970–A limnological study of the New Braunfels-Gonzales stretch of the Guadalupe River. M.A.

214. Peters, M.S., 1975–The Ephemeroptera nymphs of the Guadalupe River basin, Texas. M.S. (In progress).

214a Phillips, T.R., 1975–Seasonal succession of plankton in a cooling reservoir. M.S. (In progress)

214b Prentice, J.A., 1973–Validation of aging techniques for large-mouth bass, *Micropterus salmoides* and channel catfish, *Ictalurus punctatus*, in Central Texas farm ponds. M.S.

214c Ralph, J.J., 1975–Adsorption and desorption of cations in aquatic sediments. M.S. (In progress)

214d Rhodes, C., 1973–The influence of oxygen concentrations on the activity and survival of Hydracarina. M.A.

214e Schenck, John, 1975–Ecology of the fountain darter, *Etheostoma fonticola* (Osteichthyes: Percidae). M.S.

214f Strenth, N.E., 1970–A morphological study of the post-embryonic stages of *Eulimnadia texana* (Conchostraca, Crustacea). M.A.

214g Tatum, J.W., 1970–Physico-chemical limnology and chlorophyll "a" of the New Braunfels-Gonzales, Texas, stretch of the Guadalupe River. M.A.

214h Warren, J., 1975–Subterranean aquatic organisms as potential indicators of groundwater pollution. M.S. (In progress)

214i Woerner, H., 1971–Multiple regression analysis of 19 variables influencing eutrophication in a 153-km stretch of the Guadalupe River, Texas. M.A.

214k Young, W.J., 1971–The influence of impoundment and thermal stratification in Canyon Reservoir on the physicochemical limnology and chlorophyll a of the Guadalupe River, Texas. M.A.

STEPHEN F. AUSTIN STATE UNIVERSITY

215. Adler, P.M., 1972–A study of eutrophication in upper Sam Rayburn Reservoir. M.S.

216. Alford, H.W., 1950–Bacteriological analysis of rural water supplies in Nacogdoches County, Texas. M.S.

217. Allard, D.W., 1974–Zooplankton population dynamics at Sam Rayburn Reservoir. M.S.

218. Bachtel, H.J., 1940–Freshwater mussels of East Texas. M.S.

219. Bailey, T.F., 1970–Food habits of adult *Pomoxis annularis* and *Poxomis nigromaculatus* in Sam Rayburn Reservoir, Texas. M.S.

220. Bowers, J.H., 1962–Food habits of water snakes, genus *Natrix*, in Bowie and Red River Counties, Texas. M.S.

221. Castleberry, W.B., 1942–A biological investigation of the common fish of Central East Texas. M.S.

222. Cox, D., 1975–Benthic macroinvertebrates as indicators of water quality in the Angelina River, Texas. M.S. (In progress)

223. Dawson, J.E., 1973–The influence of Sam Rayburn Reservoir on the water quality of the Angelina River and Attoyac Bayou. M.S.

224. Dickens, F.A., 1950–A distributional study of fishes in the Nacogdoches area. M.S.

225. Foster, R.E., 1972–A survey of aquatic beetles in the city of Nacogdoches, Texas, and environs. M.S.

226. Hale, J.N., 1973–Existing water quality for the proposed site of Lake Nacogdoches. M.S.

227. Hanicak, E., 1946–Black bass of East Texas. M.S.

228. Harris, M.A., 1973–Analysis of the Trinity River coliform and streptococcus bacteria as indicators of fecal pollution. M.S.

229. Harry, D.N., 1974–A limnological comparison of selected Texas reservoirs. M.S.

230. Johnson, P.D., 1975–Analysis of water chemistry in Striker Reservoir. M.S. (In progress)

231. Lock, J.T., 1967–The minimal dissolved oxygen requirements of *Ictalurus punctatus* and *Ictalurus melas*. M.S.

232. Mulvihill, S.R., 1976–Physico-chemical limnology of the Angelina River, Texas. M.S.

233. Riley, M., 1975–Some of the effects of thermal discharges on benthic macroinvertebrates in a cooling reservoir. M.S.

234. Rodgers, G.D., 1976–Comparison of zooplankton communities in Murval Reservoir and Striker Reservoir. M.S.

235. Rodgers, J.S., 1967–Interspecific hybridization in sunfishes (*Lepomis*) of Eastern Texas. M.S.

236. Rudy, K.C., 1975–The effects of hot water effluents from an electric generating plant on phytoplankton primary productivity. M.S.

237. Snelgrove, R.E., 1975–A survey of organochlorine pesticide residues in the Angelina River and Attoyac Bayou. M.S. (In progress)

238. Sniffen, R.P., 1973–A linear water quality study of Bayou Lanana from Nacagdoches, Texas, to the Angelina River. M.S.

239. Underwood, H.T., 1975–A comparative study of the parasitism of *Micropterus salmoides*, large-mouth bass, in two arms of Sam Rayburn Reservoir. M.S.

240. Wahrer, R., 1976–The periphyton community of Striker Reservoir. M.S.

241. Walker, W.T., 1973–Physical and chemical limnology of the Trinity River, Texas, and the effects of impoundment upon water quality. M.S.

242. Whitman, R.L., 1973–Survey of pesticide residues in sediments from the Upper Trinity River Basin, Texas. M.S.

243. Wood, J.M., 1972—Feeding habits of large-mouth bass, *Micropterus salmoides* (Lacepede), from the headwaters of Toledo Bend Reservoir. M.S.

TEXAS A & I UNIVERSITY

244. Perez, R.G., 1971—Relationships between phytoplankton and chlorophyll standing crop in two permanent freshwater ponds in a marine supratidal environment. M.S.

245. Pineda, P.H.A.K., 1975—A study of fishes of the lower Nueces River. M.S.

246. Serota, T., 1971—Relationships between primary productivity and chlorophyll standing crop in two permanent freshwater ponds in a marine supratidal environment. M.S.

TEXAS A & M UNIVERSITY

247. Adams, C.S., 1935—Annual rainfall and runoff relations on the Trinity River watershed above, Dallas, Texas. M.S.

248. Albaugh, D.W., 1973—Life histories of the crayfishes *Procambarus acutus* and *Procambarus hinei* in Texas. Ph.D.

249. Allard, D., 1976—Productivity of littoral microcrustaceans in a South Texas pond. Ph.D. (In progress)

250. Allison, R.C., 1970—Algal uptake of selected organic compounds. Ph.D.

251. Anderson, A.J., 1974—A method for determining the optimization of alternate uses of river oxbows. Ph.D.

252. Bedford, W.B., 1972—The physiological ecology of the estuarine clam *Rangia cuneata* (Gray). Ph.D.

253. Bedinger, Jr., C.A., 1974—Seasonal changes in condition and biochemical constituents of the brackish water clam *Rangia cuneata* (Gray). Ph.D.

254. Bellamy, J.D., 1941—Organography of the *Notropis potteri* (Hubbs). M.S.

255. Bennett, J., 1975—Polarized light reception in turtles. M.S. (In progress)

256. Berti, A.L., 1939—A survey of anopheline larvae and breeding places at College Station, Texas. M.S.

257. Brooks, J.M., 1970—The distribution of organic carbon in the Brazos River basin. M.S.

258. Brown, P.J., 1974—An investigation of the toxin of *Microcystis aeruginosa*: Effects of varying the concentration of selected salts upon toxicity and the effects of the toxin on some physiological parameters. Ph.D.

259. Burchell, L.C., 1974—Rural reservoir recreation impact: perceptions of knowledgeable leaders. Ph.D.

260. Calnan, T., 1975—Systematics and distribution of the metazoan parasites in the Unionidae in the Navasota River, Texas. M.S.

261. Calvin, T.L., 1974—An ecological study of the amphibians and reptiles of the Navasota River, Texas. M.S.

262. Carillo, S.J., 1941—A survey of anopheline larvae in 39 stock tanks in Brazos County, Texas. M.S.

263. Champ, M.A., 1973—Organic and inorganic carbon cycles in a pond ecosystem. Ph.D.

264. Clements, G., 1975—Relation of phytoplankton population to pond fertilization. M.S. (In progress)

265. Colle, D.E., 1976—The food habits of three centrachids in a Central Texas farm pond. M.S.

266. Cowan, W.L., 1933—A study of the rainfall and the stream flow of the Navasota River system. M.S.

267. Crew, Henry, 1970—A numerical model of the dispersion of a dense effluent in a stream. Ph.D.

268. Cole, D.R., 1973—The effects of DDT and other insecticides on accumulation, growth, and photosynthesis in *Chlorella pyrenoidosa* (CHICK). Ph.D.

269. Daen, Philip, 1976—Polarized light reception in fish and sharks. M.S. (In progress)

270. Davy, F.B., 1973—The role of chemoreception in teleost locomotion behavior as affected by a pesticide. Ph.D.

271. Drier, Thomas, 1976—Use of the scanning electron microscope in quantitative estimates of aquatic bacterial populations. M.S. (In progress)

272. Dry, Eddie Maurice, 1975—An economic analysis of the Brazos River waterway. Ph.D.

273. Dziuk, Larry J., 1971—A study of pesticide residue levels and insecticide resistance in selected aquatic organisms occurring around the Bryan-College Station agricultural production areas. M.S.

274. Evans, J.W., 1975—Longitudinal distribution of fish in an East Texas stream. M.S. (In progress)

275. Farrell, W.J., 1965—Distribution of the Cladocera of Brazos County. M.S.

276. Gallaher, W.B., 1974—A limnological investigation of the relationship between the Navasota River, Texas, and a selected floodplain. Ph.D.

277. Gauntt, W.C., 1939—Studies of winter hibernation of *Anopheles* larvae at College Station, Texas. M.S.

278. Glaze, F.M., 1973—An analysis of rural subdivision development on selected reservoirs in the Trinity River Basin Texas. Ph.D.

279. Gleastine, Billy W., 1974—A study of the cichlid *Tilapia aurea* (Steindachner) in a thermally modified Texas reservoir. M.S.

280. Gillard, R.M., 1974—Distribution, abundance, and species diversity of macrobenthic and meiobenthic invertebrates in relation to Houston Ship Channel pollution in upper Galveston Bay and Tabbs Bay, Texas. Ph.D.

281. Hammerschmidt, P.C., 1974–The use of fish in cages as biological monitors of the quality of water passing through a power plant. M.S.

282. Harry, David, 1976–Factors contributing to variation in productivity among Texas reservoirs. Ph.D. (In progress)

283. Hodson, R.G., 1973–A comparison of occurrence and abundance of fishes within three Texas reservoirs which receive heated water discharges. Ph.D.

284. Hutton, W.S., 1970–A quantitative and qualitative survey of benthal deposits contained in the Houston Ship Channel. M.S.

285. Helm, J.C., 1972–Methodology for systematic planning of regional water management. Ph.D.

286. Islam, M.A., 1972–The effect of temperature on reproduction of the red shiner *Notropis lutrensis* (Baird and Girard). Ph.D.

287. Johnson, K.W., 1973–Occurrence and abundance of fishes in the intake and discharge areas of the Cedar Bayou power station before and during the first year of plant operation. Ph.D.

288. Joyce, J.A., 1973–The effects of stocking density and feeding rate on the growth of channel catfish *Ictalurus punctatus* (Rafinesque), in floating areas. M.S.

288a Jones, T.L., 1975–Species composition, distribution and abundance of macrozooplankton in the intake and discharge areas after construction and operation of the Cedar Bayou electric power station. M.S.

288b Jackson, W.B., 1974–Distribution and abundance of shrimps, crabs, and fishes in the cooling lake of the Cedar Bayou electric power station. M.S.

289. Kirk, William, 1976–The effect of varied Ca, Fe, Mg, and pH on growth and taxonomic considerations for four desmid species. Ph.D. (In progress)

290. Klussmann, W.G., 1973–An evaluation of the utilization of anhydrous ammonia as fisheries management technique in ponds. Ph.D.

291. Landry, Andre M., 1971–Number of individuals and injury rates of economically important fish passing through the P.H. Robinson generating station. M.S.

292. Lehmberg, Richard Verne, 1974–Nutrient limitation determinations for a Texas watershed. Ph.D.

292a Linder, D.R., 1974–The culture of marine fish and their use as biological monitors of water quality in ponds receiving heated discharge from a power plant. M.S.

293. Littleton, G., 1976–Growth characteristics of the Unionidae found in a stretch of the Navasota River. M.S. (In progress)

294. Lock, J.T., 1971–The effects of fertilization on the fishes of a Central Texas farm pond. Ph.D.

295. Lukins, D., 1976–Ephontic algal productivity in an East Texas stream. Ph.D. (In progress)

296. Malar, T., 1972–The locomotion and orientation of goldfish in a sound field. Ph.D.

297. Malouf, J.B., 1974–The development of a mathematical model to predict runoff water quality from watersheds. Ph.D.

298. Marquis, S., 1976–Survey of the *Mallomonas* (Chrysophyta) found in South Central Texas. M.S. (In progress)

299. McBee, Jr., J.T., 1975–Species composition, distribution, and abundance of macrobenthic organisms in the intake and discharge areas before and after the construction and operation of the Cedar Bayou electric power station. Ph.D.

300. McCullough, Jr., J.D., 1970–Carbon fixation and community dynamics of a phycoperiphyton community. Ph.D.

301. McCullough, M.M., 1971–A study of the juvenile fish fauna associated with the cooling water of a steam electric generating station. M.S.

302. Medlen, Ammon Brown, 1952–Studies on the development of *Gambusia affinis*. Ph.D.

303. Meyers, D.G., 1973–Comparative physical limnology of farm ponds in South Central Texas. M.S.

304. Mitchell, T.M., 1970–The dispersion of dense effluent from an inclined jet discharging into still fluid. M.S.

305. ————, 1974–A parametric model for a dense plume near a stream bed. Ph.D.

306. Moore, J., 1973–The effect of water quality on chemical and physical properties of a Hoban silty clay loam soil in situ. Ph.D.

307. Moskovito, G., 1961–Studies on diatom-bacteria interactions. Ph.D.

308. Moulton, B.A., 1976–Limnological study of a small watershed draining into the Navasota River. Ph.D. (In progress)

309. Murrell, J.L., 1973–The intensive cage culture of channel catfish *Ictalurus punctatus* (Rafinesque), in the intake and discharge canals of a steam electric generating station, Trinidad, Texas. M.S.

310. Parmer, David Lee, 1970–Some mechanisms of organism limitations in the inland Houston ship channel. M.S.

311. Petrocelli, S.M., 1973–The interactions of a chlorinated hydrocarbon insecticide among the water, sediments and biota in an estuarine system. Ph.D.

312. Phantumvanit, D., 1974–Simulation and statistical quality control for water pollution abatement. Ph.D.

313. Phelps, R., 1976–Repopulation dynamics of the macrobenthos in a ripple area of the Little Brazos River, Texas. Ph.D. (In progress)

314. Prather, J.E., 1976–The influence of thermal inflow on the distribution and condition of freshwater drum in Lake Trinidad, Texas. M.S. (In progress)

314a Quarberg, D.M., 1974–Report on the culture of brown shrimp, *Penaeus aztecus*, in ponds receiving thermal effluent from a power plant. M.S.

315. Ramsey, D.B., 1973–The effects of a single application of fertilizer on the fish population of Post Oak Lake. M.S.

316. Rand, G., 1976–Effect of pesticides on fish locomotion. M.S. (In progress)

317. Rennie, T.H., 1975–Zooplaknton studies in the Cox Bay, Texas, area before and during early operation of an electric power plant. Ph.D.

318. Respess, Richard O., 1970–Seasonal variations in selected physical and chemical conditions of a small impoundment in Brazos County, Texas. M.S.

319. Rozenburg, E.R., 1970–The composition and distribution of the fish fauna of the Navasota River. M.S.

320. Rubee, Peter, 1976–Behavioral studies in catfish. M.S. (In progress)

321. Runnels, W.C., 1969–Relationships between planktonic epiphytic and epipelic diatom populations in selected ponds of Brazos County, Texas. Ph.D.

322. Reed, Joel R., 1972–Social and environmental characteristics of an urban river recreation system. M.S.

323. Sarker, M.A.L., 1970–Patterns of feeding of the bluegill sunfish *Lepomis macrochirus* (Rafinesque), in two heated reservoirs of Texas. Ph.D.

324. Schwebel, M.D., 1973–Remote measures of turbidity and chlorophyll through aerial photography. M.S.

325. Serns, S.L., 1972–Age, growth, and condition of bluegill sunfish *Lepomis macrochirus* (Rafinesque), in four heated reservoirs in Texas. M.S.

326. Strenth, N.E., 1974–A review of the systematics and zoogeography of the freshwater species of *Palamonetes heller* (Crustacea, Decapoda) of North America. Ph.D.

327. Smith, Francis William, 1973–A study of waterfowl habitats, populations and fluctuations in the lower Trinity River and Upper Trinity Bay, Texas. M.S.

328. Schwebel, J.A., 1973–Hormonal control of growth and development in *Lemna minor* L., with special emphasis on the role of abscisic acid (ABA). M.S.

329. Timms, A.M., 1972–Some aspects of intraspecific communication and of the role of vision in the control and modulation of locomotor behavior of fish. Ph.D.

330. Wardle, W.J., 1974–A survey of the occurrence, distribution and incidence of infection of helminth parasites of marine and estuarine Mollusca from Galveston, Texas. Ph.D.

331. Waxman, J.B., 1974–The locomotor behavior of fish in response to a subacute concentration of copper alone and in combination with other environmental factors. Ph.D.

332. Westlake, Garson F., 1973–Some effects of thyroxine on locomotor behavior in fish. Ph.D.

333. Whitaker, R.E., 1974–Drag coefficient at hurricane wind speeds as deduced from the numerical simulation of dynamic water level changes in Lake Okeechobee. Ph.D.

333a Wiesepape, L.M., 1974–Thermal resistance and acclimation rate in young white and brown shrimp, *Penaeus setiferus* Linn. and *Penaeus aztecus* Ives. Ph.D.

334. Wood, C.E., 1969–Relationship between primary productivity ($C^{14}$ method) and phytoplankton in a mesotrophic lake. Ph.D.

335. Yeh, C.F., 1971—Mark and recovery estimates of fish populations in three heated reservoirs of Texas. M.S.

336. ————, 1972—Population studies of selected fishes in three heated reservoirs in Texas. Ph.D.

337. Zengerle, M.W., 1972—Age, growth, and condition of white crappie, *Pomoxis annularis* (Rafinesque) in Lake Nasworthy, Texas, a reservoir receiving a heated effluent. M.S.

TEXAS CHRISTIAN UNIVERSITY

338. Brooks, A.W., 1928—Freshwater Cladocera of North Texas. M.S.

339. Bryan, J., 1974—The distribution of benthic organisms in Nueces Bay, Texas, with regard to the effects of thermal loading and hurricane Fern. M.S.

340. Cappel, S.M., 1970—A comparative study of the net plankton populations in the tributaries and the main reservoir of Benbrook Lake, Tarrant County, Texas. M.S.

341. Clavin, S., 1975—The ichthyofauna of Benbrook Lake. M.S.

342. Coldiron, Donna Lee Roe, 1976—Biology: Some aspects of the biology of the exotic mollusk *Corbicula* (Bivalvia: Corbiculidae). M.S.

343. Coolridge, J., 1975—Acute toxicity of the herbicide Diquet to minnows.

344. Forsyth, J.W., 1937—Ecology of sewage filter bed with special reference to Psychoda flies. M.S.

345. Froehlich, K., 1975—Acute toxicity of organophosphorus insecticide, Malathion to the fathead minnows (*Pimephales promelas*) (Rafinesque) and golden shiners (*Notemigonus crysoleucas*) (Mitchell) M.S.

346. Green, D., 1968—Food and feeding habits of the white crappie *Pomoxis annularis* (Rafinesque) in Benbrook Lake, Texas. M.S.

347. Gruninger, T., 1975—Macroparasites of certain fishes of Eagle Mountain Lake. M.S.

348. Gunn, F.A., 1953—A limnological investigation of a freshwater impoundment in Wise County, Texas. M.S.

349. Hawley, J.B., 1926—A preliminary study of the microscopic life in Texas waters. M.S.

350. Holland, J.L., 1954—Geological investigations of Honey Creek watershed. M.S.

351. Jones, D.E., 1939—A limnological survey of Lake Como. M.S.

352. Kerby, H., 1966—Life history of largemouth bass, *Micropterus salmoides*, (Lacepede) in Benbrook Lake, Tarrant County, Texas. M.S.

353. Lawrence, J.L., 1965—Parasites of five fishes from Benbrook Lake, Tarrant County, Texas. M.S.

354. Mahon, M.F., 1928—The Phylum Rothelminths in North Texas waters. M.S.

355. Mauldin, V.L., 1972—The bivalve Mollusca of selected Tarrant County, Texas, reservoirs. M.S.

356. Moore, W.S.L., 1971–Trace metal concentrations in water and sediments of the Trinity River system, Tarrant County, Texas. M.S.

357. Paxton, J.E., 1973–A seasonal study of diurnal physico-chemical changes in a small pond in Tarrant County, Texas. M.S.

358. Pettitt, J.M., 1973–Net plankton population of Eagle Mountain Lake, Tarrant County, and Possum Kingdom Lake, Palo Pinto County, Texas. M.S.

359. Pruett, J., 1975–The ichthyofauna of Eagle Mountain Lake. M.S.

360. Rose, D.L., 1941–A limnological investigation of the Trinity River at Ft. Worth. M.S.

361. Sale, C.M., 1957–Geology along the Clear Fork of the Trinity River southwest of Ft. Worth, Texas, including Benbrook Lake. M.S.

362. Simmons, Barbara Hudson, 1976–Biology: Toxicity of selected industrial waste effluents to channel catfish eggs and fry and largemouth bass fry as determined through bioassay. M.S.

363. Taylor, R., 1975–Water quality of Sycamore Creek, Ft. Worth, Texas: A non-point pollution source. M.S.

364. Ten Eyck, J., 1975–Organic fractionization and selected trace metal content of sludges. M.S.

TEXAS TECH UNIVERSITY

365. Ali, M.M., 1970–Unlined treated sewage storage ponds as sources of nitrate pollution of the Ogallala. M.S.

366. Anderson, J.F., 1973–Variation of urban runoff quality with duration and intensity of storms. M.S.

367. Austin, T.A., 1971–A simulation model for the urban runoff process. Ph.D.

368. Brownlee, R.C., 1970–Pollution of storm runoff from a small residential watershed. M.S.

369. Carter, C.K., 1973–The effect of irrigation demand forecasting on the optimal operation of the Elephant Butte-Caballo Reservoir system. M.S.

370. Chapman, S., 1974–Nitrogen mass balance determination for simulated wastewater land-spreading operations. M.S.

371. Chitwood, J.R., 1965–Hydraulics of groundwater flow. M.S.

372. Compton, J.L., 1975–Diatoms of the Lubbock Lake site, Lubbock County, Texas. M.S.

373. Cook, B.C., 1973–The effects of recreation pool size on irrigation and power generation at Elephant Butte Reservoir. Ph.D.

374. Foerster, E.P., 1965–A study of the effects of detergents on seepage rates. M.S.

375. Forehand, C.E., 1966–Fate of pesticides applied to lake beds and surrounding water sheds. M.S.

376. Griffin, D.G., 1962–A population study of *Chara zeylanica* in Texas, Oklahoma and New Mexico. M.S.

377. Hejl, Henry R., 1964–A study of the time of concentration for small natural watersheds. M.S.

378. Henderson, G.T., 1961–Some factors affecting oospore germination in *Chara zeylanica* (Willdenow). M.S.

379. Keenum, B.J.B., 1975–Comparisons of approximate F tests and a procedure for the development of an alternative. M.S.

380. Knowles, T.R., 1971–The development of a computer model for confined and unconfined aquifers. M.S.

381. Knowles, T.R., 1972–The development of a computerized procedure to determine aquifer parameters. Ph.D.

382. Lee, M.M., 1965–Percolation of salt water in an unconfined aquifer. M.S.

383. Lovell, T.L., 1969–A comparison of system designs and operations for the transmission of large volumes of water to West Texas. M.S.

384. Malone, C.R., 1965–Dispersal of freshwater gastropods by water birds. M.S.

385. Mocek, M.J., 1971–A study of the compatibility of conjunctive use and Texas groundwater law. M.S.

386. Pettit, G.M., 1974–Evaluation of parameters for the production of water availability from ungaged watersheds in Texas. M.S.

387. Rauschuber, D.G., 1972–A mathematical model to correlate rainfall and runoff. M.S.

388. Roberts, D.W., 1970–Mathematical model: A tool for water management. M.S.

389. Stephens, D.W., 1966–The economics of a constructed pit for groundwater recharge on the high plains of Texas. M.S.

390. Thompson, G.B., 1974–Variation of urban runoff quality and quantity with duration and intensity of storms. M.S.

391. Ward, C.R., 1964–Ecological changes in modified playa lakes with special emphasis on mosquito production. M.S.

392. Webster, R.D., 1973–Development and application of coaxial correlation techniques to predict runoff from small watersheds. M.S.

393. Williams, N.R., 1969–Population ecology of *Natrix harteri*. M.S.

394. Winn, W.T., 1973–A pilot study of the Canyon Lakes project. M.S.

395. Yeager, C.C., 1965–A study of a salt-water wedge trapped in a freshwater aquifer. M.S.

TRINITY UNIVERSITY

406. Albright, P.N., 1952–Contributions to the knowledge of the Odonata of Texas with particular attention to the area surrounding San Antonio. M.S.

407. Giesler, R.S., 1972–A comparison of the benthic macroinvertebrates of Calaveras and Braunig Lakes. M.S.

408. Smalley, H.E., 1956–The mosquito fauna (Diptera-Culicidae) of San Antonio, Texas. M.S.

UNIVERSITY OF OKLAHOMA

409. Ausburn, B.E., 1961–Subsurface disposal of natural brines in western Oklahoma and northern Texas. M.S.

410. Baglin, Raymond E., Jr., 1976–Age and growth characteristics of white bass *Morone chrysops* (Rafinesque) in Lake Texoma, Oklahoma. Ph.D.

411. Campbell, J.W., 1955–A description of *Lissorchis gullaris* n. sp. (Trematoda: Lissorchilidae) from the buffalo fish of Lake Texoma, Oklahoma. M.S.

412. Dowell, V.E., 1956–Activity patterns and distribution of the fishes in the Buncombe Creek Arm of Lake Texoma, Oklahoma. Ph.D.

413. Echelle, A.A., 1966–The food of young-of-year gars, *Lepisosteus*, in Lake Texoma with notes on spawning and development. M.S.

414. Grinstead, B.G., 1965–The vertical distribution of the white crappie, *Poxomis annularis*, in the Buncombe Creek Arm of Lake Texoma. M.S.

415. Houser, A., 1958–A study of the commercial fishery of Lake Texoma. M.S.

416. Kilpatrick, E.B., 1959–Seasonal cycle in the gonads of the white bass (*Roccus chrysops*), in Lake Texoma, Oklahoma. Ph.D.

417. McDaniel, J.S., 1961–A survey of the parasites of the genus *Lepomis* (Centrarchidae) from the Buncombe Creek Arm of Lake Texoma. M.S.

418. Martin, J.M., 1952–Age and growth of the goldeye, *Hiodon alosoides* (Rafinesque) of Lake Texoma, Oklahoma. M.S.

419. Mense, J.B., 1967–Ecology of the Mississippi silversides, *Menidia audens* Hay, in Lake Texoma. M.S.

420. Moser, B.B., 1968–Food habits of the white bass in Lake Texoma with special reference to the threadfin shad. M.S.

421. Riggins, M.A., 1953–A study of the helminth parasites of certain shore birds from Lake Texoma, Oklahoma. M.S.

422. Saunders, R.P., 1959–A study of the food of the Mississippi silversides *Menidia audens* Hay, in Lake Texoma. M.S.

423. Shelton, W.L., 1972–Comparative reproductive biology of the gizzard shad, *Dorosoma cepedianum* (Lesueur), and the threadfin shad, *Dorosoma petenense* (Gunther), in Lake Texoma, Oklahoma. Ph.D.

424. Sublette, J.E., 1953–The ecology of the macroscopic bottom fauna in Lake Texoma. Ph.D.

425. Szalwinski, A.A., 1967–The highland lakes of Texas: a recreational study. M.A.

426. Taber, C., 1969–The distribution and identification of larval fishes in the Buncombe Creek Arm of Lake Texoma with observations on spawning habits and relative abundance. Ph.D.

427. Whiteside, B.G., 1964–Biology of the white crappie, *Poxomis annularis*, in Lake Texoma. M.S.

428. Wilson, C., 1950–Age and growth of the white crappie *Poxomis annularis* (Rafinesque) in Lake Texoma, Oklahoma. M.S.

UNIVERSITY OF TEXAS AT ARLINGTON

429. Aldridge, D., 1975–Biometrics and population dynamics of *Corbicula manilenais* in Lake Arlington. M.S. (In progress)

430. Barnett, J., 1974–Littoral macrobenthos of Lake Arlington. M.S.

431. Bird, J.L., 1975–Relations of zooplankton to water stratification due to density differences resulting from thermal effluents. M.S. (In progress)

432. Carr, L., 1972–The effect of a thermal outfall on the phytoplankton of Lake Arlington. M.S.

433. Hall, F., 1972–Species diversity of the benthic macroinvertebrates inhabiting a reservoir receiving a heated effluent. M.S.

434. Karbach, A., 1975–Ecological survey of insular plants and vertebrates of Lake Arlington. M.S. (In progress)

435. Kelly, M., 1975–Fishes of the headwaters of the Trinity River. M.S. (In progress)

436. Parker, F.R., 1971–Reduced metabolic rates in fishes as a result of induced schooling. M.S.

437. Rutledge, C.J., 1975–Fishes of Lake Arlington. M.S. (In progress)

438. Smith, M.D., 1973–Water quality survey of Benbrook Lake. M.S.

439. Speegle, M., 1973–Limnology and ichthyology of Lake Fairfield, Texas. M.S.

440. Thompson, W., 1972–Potential storm water pollution from downtown Dallas. M.S.

441. Tommey, W.H., 1975–Fishes of Monticello Reservoir. M.S. (In progress)

UNIVERSITY OF TEXAS AT AUSTIN

442. Aguirre, Jorge, 1967–Nitrification and denitrification in a model waste stabilization pond. M.S.

443. ————, 1971–Influence of the anaerobic sludge layer location on facultative waste stabilization pond performance. Ph.D.

444. Archibald, Patricia A., 1969–A study of the algal flora of bogs with special reference to the genus *Chlorococcum*. Ph.D.

445. Baker, Ailsie F., 1969–Taxonomic studies in the Oscillatoriaceae. Ph.D.

446. Barnhill, Betty Louise, 1970–Aggressive interactions in reproductively active *Cyprinodon variegatus*. M.S.

447. Batterton, John Clyde, Jr., 1967–Studies of the phosphorus-deficient state in the blue-green alga *Anacystis nidulans*. M.A.

448. ————, 1970–A study of halotolerance in blue-green algae. Ph.D.

449. Bechtel, Timothy Joseph, 1970–Fish species diversity indices as pollution indicators in Galveston Bay, Texas. M.S.

450. Beyers, Robert John, 1962–The metabolism of twelve aquatic laboratory microecosystems. Ph.D.

451. Bhagat, Suriner Kumar, 1966–Transport of nitrosylruthenium in an aquatic environment. Ph.D.

452. ————, 1962–Estimating algal concentrations. M.S.

453. Birke, Lawrence Edward, Jr., 1968–Development of a blue-green algal assay for vitamin B-12 application to an ecological study of the San Antonio estuary. M.S.

454. Bischoff, Harry W., 1963–I. The soil algal flora of Enchanted Rock, II. *Thorea riekei* sp. nov. and related species. Ph.D.

455. Bishop, Neil Erasmus,1975–Studies on mixing and heat exchange in aerated lagoons. Ph.D.

456. Blystone, Robert Vernon, 1968–The hemoglobin of *Daphnia magna* (Straus). M.S.

457. Bohmfalk, Clyde Edward, 1971–Differentiation in temperate and tropical zone populations of *Typha* under transplant garden and controlled photoperiod and thermoperiod conditions. Ph.D.

458. Bolch, William Emmett, Jr., 1964–Behavior of ruthenium in algal environments. M.S.

459. Brackenridge, Genie, 1968–The ultraviolet spectral analysis of coumarins. M.S.

460. Brock, David A., 1975–Niche response structure of competition between *Scenedesmus obliquus* and *Chlorella vulgaris*. M.S.

461. Bronaugh, Richmond Lee, 1950–Geology of Brazos River terraces in McLennan County, Texas. M.A.

462. Brown, Richard Malcolm, Jr., 1964–A comparative study of the algal genera *Tetracystis* and *Chlorococcum*. Ph.D.

463. Brown, Billy Austin, 1975–Exchange of nitrogen and phosphorus with sediments of the brackish water marshes of Lavaca Bay, Texas. M.S.

464. Buchan, Glenn Carl, 1972–A cybernetic approach to thermal pollution decision-making. Ph.D.

465. Bullock, Charles William, 1972–Measurement and prediction of abnormal reservoir operations on Lake LBJ's water quality. M.S.

466. Burch, Marvin Chandler, 1950–A history of the lower Trinity River region to 1836. M.S.

467. Cain, Brother Joseph R., 1963–The morphology, taxonomy and physiology of certain *Chlamydomonas*-like algae. Ph.D.

468. Calhoun, Sam Harlan, 1970–An investigation of the oxygen balance in the Colorado River, Lake Austin, Texas. M.S.

469. Carlisle, Clara, 1924–The rate of disappearance of chlorine in drinking water. M.A.

470. Carr, Roseanne, 1972–Field and culture studies of the diatom genus *Biddulphia* in the Gulf of Mexico. M.S.

471. Carroll, William Erwin, Jr., 1972–Hypolimnetic dissolved oxygen simulation model. M.S.

472. Chambers, John Edward, 1962–Development of a polarographic system for the measurement of oxygen exhange in algal suspensions. M.S.

473. Chavez, Pedro Islas, 1973–Variation in sesquiterpene lactones within a Trinity River, Texas, population of *Xanthium strumarium*. M.S.

474. Cech, Joseph Jerome, Jr., 1970–Respiratory responses of the striped mullet, *Mugil cephalus*, to three environmental stresses. M.S.

475. Chen, Tony Gwokwong, 1972–Selective withdrawal at Lake Livingston. M.S.

476. Clark, Thomas Phillips, 1972–Hydrology, geochemistry, and public health aspects of environmental impairment at an abandoned landfill near Austin, Texas. M.S.

477. Clark, George Gregory, 1973–Evaluation of loading on water quality attributable to non-point sources. M.S.

478. Cobb, Howell Dee, 1963–Studies on nitrogen fixation in a blue-green alga. Ph.D.

479. Collier, Robert Gressett, 1939–A survey of the water fauna of Houston and its vicinity and an analytical study of its use in teaching biology. M.Ed.

480. Cooper, B.D., 1954–The feeding habits of the largemouth bass (*Micropterus salmoides*). M.S.

481. Cooper, David Clayton, 1970–Responses of continuous-series estuarine microecosystems to point source input variations. Ph.D.

482. Cox, E.R., 1966–Taxonomic, morphological and physiological studies of the algal genus *Stigeoclonium*. Ph.D.

483. Cramer, Marian Lenore, 1952–Studies on growth and photosynthesis of *Euglena* with emphasis on the replacement of $CO_2$ in metabolism. Ph.D.

484. Crawley, Richard Alvin, 1969–Flood effects on Sanderson Creek, Trans-Pecos Texas, June 11, 1965. M.S.

485. Crow, Larry Isaac, 1974–A numerical study of the velocity and temperature fields in a stratified reservoir. M.S.

486. Crowley, Dennis John, 1971–Evaluating the effects of return flows on stream quality in terms of total dissolved solids. M.S.

487. Cumming, Robert Bruce, 1964–Cytogenetic studies in the order Odonata. Ph.D.

488. Darnell, Paul Edward, 1972–Upflow filtration of waste stabilization pond effluent. M.S.

489. Davenport, James B., 1975–Phytoplankton succession and the productivity of individual algal species in a Central Texas reservoir. M.S.

490. Davidson, F.F., 1941–Taxonomy of Texas Zygnemataceae. Ph.D.

491. Dawson, Anita Joan, 1976–The role of plants in nutrient exchange in the Lavaca Bay brackish marsh system. M.S.

492. Deason, Temd Robert, 1960–Exploratory studies of Texas soil algae. Ph.D.

493. Delco, A., 1962–Reproductive behavior and sexual isolation mechanisms between two sympatric cyprinid fishes. M.S.

494. Dharmikarak, Sawasdi, 1957–Coagulation of Lake Austin water. M.S.

495. Diehl, W.H.S., 1959–Peak discharge predictions for Waller Creek at 23rd Street at Austin, Texas. M.S.

496. Drewry, George Earl, 1962–Some observations of courtship behavior and sound production in five species of *Fundulus*. M.S.

497. ————, 1967–Studies of relationships within the family Cyprinodontidae. Ph.D.

498. Drynan, Walter Ronald, 1956–Methods of concentrating algae. M.S.

499. ————, 1961–Radioactivity in surface waters: a statistical approach to establishing base levels. Ph.D.

500. Duke, M.E.L., 1967–A production study of a thermal spring. Ph.D.

501. Dykstra, Richard F., 1966–An investigation of some algae of the Texas Gulf Coast. M.A.

502. de la O, Ernesto Espino, 1968–Sulfide production in waste stabilization ponds. Ph.D.

503. Eddleman, Charles Douglas, 1965–A morphological study of the early instars of mosquito larvae. M.S.

504. Edwards, Linda Kay, 1969–Some non-aquatic epiphytic and lithophilous algae. M.S.

505. Edwards, Peter, 1969–Field and cultural studies of the seasonal periodicity of growth and reproduction of selected Texas marine algae. Ph.D.

506. Eley, James Henry, Jr., 1967–Enhancement of photosynthesis in *Chlorella* and a kinetic model for photosynthesis. Ph.D.

507. Elias, Robert William, 1973–The role of seagrasses and benthic algae in the biogeochemistry of trace metals in Texas estuaries. Ph.D.

508. Espey, William Howard, Jr., 1965–A study of some effects of urbanization on storm runoff from a small watershed. Ph.D.

509. Felsing, William August, Jr., 1962–$Sr^{90}$ and $Ra^{226}$ in a domestic water supply. M.S.

510. Fisher, Charles Parker, 1963–Stabilization of biosorption activated sludge in waste stabilization ponds. Ph.D.

511. Floyd, Bert Allen, 1969–The use of algal cultures to assess the effects of nutrient enrichment on the Highland Lakes of the Colorado River, Texas. M.S.

512. Fogg, John Brian James, 1973–A dissolved oxygen model for the hypolimnion of Lake LBJ. M.S.

513. Ford, Davis Lee, 1965–Kinetics of aerobic oxidation in the thermophilic range. M.S.

514. Garner, Lee Edwin, 1973–Environmental geology of the Austin area, Texas. M.S.

515. Gazda, Lawrence Paul, 1969–Land disposal of municipal solid wastes in selected standard metropolitan statistical areas in Texas. M.S.

516. Gearing, Patrick James, 1970–A study of the effect of TC-99 Pertechnetate on blue-green algae. M.A.

517. Gebhard, Thomas Granville, Jr., 1964–Streamflow frequency distributions on selected streams in Texas. M.S.

518. Gerald, Jerry Wayne, 1970–Species isolating mechanisms in the genus *Lepomis*. Ph.D.

519. Govin, Charles Thomas, Jr., 1973–Sedimentation survey, Lake Buchanan, Texas, 1973. M.S.

520. Gravel, Alan Charles, 1969–The distribution of coliform bacteria in stratified impoundments. M.S.

521. Green, George Edward, 1975–An assessment of phosphorus data collected from Lake Lyndon B. Johnson. M.S.

522. Groover, Robert Don, 1968–Experimental studies on Chlorosphaeracean and other edaphic algae. Ph.D.

523. Guaqueta, Camilo Hernando, 1973–Environmental and economic impact of pollution abatement by SIC 28 and 29. M.S.

524. Hagen, Donald Warren, 1963–Some aspects of thermal tolerance in three species of *Gambusia*. M.S.

525. Harris, James Carl, 1971–Pollution characteristics of channel catfish culture. M.S.

526. Haughton, Edward Ronald, 1970–Oxygen depletion and prediction in the hypolimnion of a southwestern reservoir. M.S.

527. Henderson, G.G., 1960–Stomach contents of Central Texas specimens of *Percina caprodes*. M.S.

528. Herman, Edward Robert, 1957–Development of design criteria for waste stabilization ponds. Ph.D.

529. Herman, William Hoffman, Jr., 1976–A continuous equation to describe bioassay results for acute exposure of fish to toxins. M.S.

530. Higgins, Robert Brown, 1969–Enzymatic method for the detection of luxury phosphorus uptake. M.S.

531. Hoffman, Larry R., 1961–Studies on the morphology, cytology and reproduction of *Oedogonium* and *Oedocladium*. Ph.D.

532. Hu, Vicky Ray-Shen, 1971–Nutrient studies in Texas impoundments. M.S.

533. Huang, George S., 1968–Scheduling of releases to meet downstream irrigation requirements. M.S.

534. Huang, Ju-Chang, 1967–Effects of toxic organics on photosynthesis reoxygenation. Ph.D.

535. Hung, Yung-Tse, 1970–Biological reaction rate constant in natural water. Ph.D.

536. Ingram, Lonnie O'Neal, 1971–Mutations and cell division in blue-green algae. Ph.D.

537. James, Stephen Nelson, 1974–An analysis of factors affecting the distribution of nekton in and around a power generating station in an estuarine area. M.S.

538. Jetton, Elden Verner, 1973–Climatology of the upper Rio Grande Basin and the development of spring runoff forecast equations. Ph.D.

539. Jones, Larry Warner, 1964–Photosynthetic enhancement phenomena in a blue-green alga, *Anacystis nidulans*. Ph.D.

540. Jurgens, K.C., 1951–The distribution and ecology of the fishes of the San Marcos River (Austin, Texas). M.S.

541. Kantz, Paul Thomas, Jr., 1966–Some South-Texas soil algae with special reference to the genus *Nostoc*. M.A.

542. —— ——, 1968–Taxonomic, morphological, and physiological studies of the algal genera *Nostoc* and *Anabaena*. Ph.D.

543. Kapraun, Donald Frederick, 1969–Field and cultural studies of the genera *Ulva* and *Enteromorpha*. Ph.D.

544. Kemp, Robert James, Jr., 1950–A comparative study of brackish water fishes. M.S.

545. Kikuchi, Noboru, 1975–A study of variational inequalities with a class of seepage flow problems. M.S.

546. Killam, Allen Page, 1955–Special effects of light on *Chlorella*. M.S.

547. Koch, Charles Thomas, 1966–Organic constituents in surface waters. M.S.

548. Kratz, William A., 1954–Studies on the physiology of blue-green algae. Ph.D.

549. Kreitler, Charles Wrightman, 1974–Determining the source of nitrate in groundwater by nitrogen isotope studies. Ph.D.

550. Krise, George Martin, Jr., 1952–A study of nitrogen excretion of ciliate protozoa. Ph.D.

552. Ledbetter, Joe Overton, 1963–Predictive techniques for water quality-inorganics. Ph.D.

553. Lee, Chin-Yuan, 1968–Macro-turbulence in wind waves. Ph.D.

554. Lennington, Richard Kent, 1975–The analysis of clustering methods as applied to biological population data. Ph.D.

555. Lindstrom, Bruce M., 1975–An analysis of niche response of Lake LBJ phytoplankton to environmental levels of light and temperature. M.S.

556. La Montagne, John Ring, 1967–A procedure for the isolation of heterocysts from blue-green alga *Anabaena cylindrica* (Fogg). M.S.

557. Laplante, Joseph Michael, 1975–Investigation of uptake characteristics of various clays for selected heavy metals. M.S.

558. Lytle, Thomas Foster, 1966–The biogeochemistry of trace metals in the near shore environment. Ph.D.

559. MacRae, John R., 1975–Some effects of temperature on the population dynamics of planktonic rotifers. M.S.

560. Martin, Floyd Douglas, 1967–Osmotic shock survival in certain cyprinodont fishes. M.S.

561. ————, 1968–Some factors influencing penetration into rivers by fishes of the genus *Cyprinodon*. Ph.D.

562. Mattox, Karl R., 1962–The taxonomy of certain ulotrichalean algae. Ph.D.

563. Matusi, Saburo, 1972–Biological accumulation of mercuric and methyl mercury by *Chlorella pyrenoidosa*. Ph.D.

564. McCarver, C.T., 1950–The effects of pollution by water purification sludge on freshwater fishes (Austin, Texas). M.S.

565. McClure, Jerry Weldon, 1964–Taxonomic significance of the flavonoid chemistry and the morphology of Lemnaceae in axenic culture. Ph.D.

566. McClure, John Robert, 1962–Analysis of the stationary and death phases of the growth curve of *Tetrahymena pyriformis*. M.S.

567. McGowen, Joseph Hobbs, 1969–Gum Hollow Fan Delta. Nueces Bay Texas: mode of development and sedimentation characteristics. Ph.D.

568. McLaurin, Banks, 1928–The silt problem in Texas. M.S.

569. McHale, John Thomas, 1965–The reduction of heavy metal salts by chloroplasts, with reference to ascorbic acid. Ph.D.

570. McNaughton, Samuel Joseph, 1964–Ecotypic patterns in *Typha* and their significance in ecosystem integration. Ph.D.

571. Melbard, Albert Roy, 1974–Inactivation of virus in bench-scale oxygenerated waste stabilization ponds. M.S.

572. Meier, W.L., 1964–Analysis of unit hydrographs for small watersheds in Texas. M.S.

573. Milliger, Larry Edward, 1965–Some soil algae from Bastrop State Park, Texas. M.S.

574. Mills, Jimmy Tom, 1972–The biology of two species of haptophycean algae (Chrysophyta). Ph.D.

575. Mims, Charles Wayne, 1969–An ultrastructural study of the life cycle of the Myxomycete *Arcyria cinerea*. Ph.D.

576. Moore, Richard Byron, 1966–Survival studies on the blue-green alga, *Anacystis nidulans*. Ph.D.

577. Moore, Harry Grady, Jr., 1969–Studies on the surplus phosphorus uptake phenomenon in algae. Ph.D.

578. Moran, Robert Edward, 1974–Trace element content of a stream affected by metal-mine drainage, Bonanza, Colorado. Ph.D.

579. Nations, M.A., 1959–Multiple correlation of rainfall and other factors to runoff. M.S.

580. Neill, William Edward, 1972–Effects of size-selective predation on community structure in laboratory aquatic microcosms. Ph.D.

581. Nelson, Robert Young, 1964–Atmospheric emissions from waste stabilization ponds. Ph.D.

582. Nokes, Mark Alan, 1972–Effects of x-irradiation on photosynthesis in *Chlorella pyrenoidosa*. M.S.

583. Palmer, Neil Meredith, 1949–An approach to the ecology of the lower Rio Grande Valley. M.S.

584. Parker, Bruce C., 1960–Environmental investigations of the relationships of certain soil algae with their associated microorganisms. Ph.D.

585. Patyrak, Richard Clark, 1972–The distribution of DDT in a laboratory soil-water system. M.S.

586. Pearson, G.P., 1955–Water resources of Texas (Austin, Texas), M.S.

587. Pearson, R.G., 1938–The aquatic oligochaeta of the Austin region. M.S.

588. Penumalli, Bhaskara Reddy, 1975–Large systems approach to water quality modeling and management. Ph.D.

589. Pessoney, George Francis, 1968–Field and laboratory investigations of Zygnemataceous algae. Ph.D.

590. Phillips, Jesse Neal, Jr., 1953–The growth rate of *Chlorella pyrenoidosa* as a function of intensity and intermittency of illumination. Ph.D.

591. Pittman, Dwight Lee, 1969–Determination of the effects of shoreline development on impoundment water quality. M.S.

592. Pocock, Mary Avis, 1966–Chemical discrimination in *Procambarus clarkii*: behavioral responses to electrolytes.

593. Poole, John Alex, 1970–A study of the death rate of *Escherichia coli* in natural waters. M.S.

594. Proffitt, Thomas Oliver, 1971–Variations in daily solar radiation at National Weather Service observing sites in Texas. M.S.

595. Pursley, Lewis Ferguson, 1970–Impoundment model for predicting water quality changes with depth in a stratified reservoir. M.S.

596. Raul, Cuellar-Chavez, 1976–Regional methodology to estimate point and nonpoint pollutional loads discharged into a stream.

597. Redford, Edward Logan, 1950–Some effects of ultraviolet radiation on *Chlorella*. M.S.

598. Reimers, Robert Stolt, 1968–A stable carbon isotope study of a marine bay and domestic sewage plant. M.S.

599. Renfro, W.C., 1958–The effect of salinity on the distribution of fishes in the Arkansas River. M.S.

600. Ruane, Richard James, 1970–Effects of environmental development on water quality and variation of water quality with depth and location-lower Highland Lakes. M.S.

601. Ruppersberger, John Sidney, 1971–Studies on nutrients released from a Trinity River sediment under oxic conditions. M.S.

602. Samejima, Hirotoshi, 1957–Heterotrophic metabolism of unicellular green algae. M.S.

603. Sauer, S.P., 1963–Multiple correlation estimates of runoff as affected by areal distribution of rainfall. M.S.

604. Sealey, Jesse Q., 1951–The biology of *Clostridium nigrificans*. Ph.D.

605. Sell, John Leroy, 1960–Study of effects of low temperature on *Anacystis nidulans*.

606. Sever, Julia Rebecca, 1968–The geochemistry of fatty alcohols in recent and ancient sediments. M.S.

607. Shen, Eugene Y.F., 1966–Morphogenetic and cytological investigations of *Chara contraria* and *C. zeylanica*. Ph.D.

608. Shull, Roger Don, 1968–Radioactivity transport in water; simulation of sustained releases to selected river environments. Ph.D.

609. Smith, Michael Alexis, 1975–Geology and trace element geochemistry of the Fort Davis area, Trans-Pecos, Texas. Ph.D.

610. Smith, Cecil Hiawatha, 1965–The synthesis of storm runoff hydrographs from unsteady, non-uniform rainfall. Ph.D.

611. Smith, Beulan Sands, 1938–A study of the algae of the Gulf Coast of Texas. M.A.

612. Smith, R.L., 1965–Morphological, taxonomic and physiological investigations of the algal genera *Eremosphaera* and *Oocystis*. M.S.

613. Sorensen, Elsie Mae Boecker, 1974–Thermal effects on the biological magnification of arsenic in green sunfish, *Lepomis cyanellus*. Ph.D.

614. Sorokin, Constantine Alexis, 1955–Studies on high-temperature algae. Ph.D.

615. Steed, David Lewis, 1967–Metabolic responses of some estuarine organisms to an industrial effluent. M.S.

616. Stern, Milton Harold, 1956–A study of generation time in *Tetrahymena pyriformis*. M.S.

617. Stewart, Jeanette Louise Kershaw, 1971–The biology of the green alga *Characiosiphon rivularis* (Iyengar). Ph.D.

618. Strawn, R.K., 1957–The influence of environment on the meristic counts of the fishes, *Etheostoma grahami* and *E. lepidum*. Ph.D.

619. Stuessy, Tod Falor, 1968–A systematic study of the genus *Melampodium* (Compositae-Heliantheae). Ph.D.

620. Sun, Elaina Si-chen Liu, 1966–Studies of East Texas soil algae with special reference to *Botrydium*. M.S.

621. Suwannakarn, Verachai, 1963–Temperature effects on waste stabilization pond treatment.

622. Thirumurthi, Dhandapani, 1966–Relative toxicity of organics to *Chlorella pyrenoidosa*. Ph.D.

623. Thomas, Dempsey L., 1968–Morphological and biochemical taxonomy of the algal genus *Protosiphon*. Ph.D.

624. Tilton, John E., 1961–Ichthyological survey of the Colorado River of Texas. M.S.

625. Tischler, Lial Frederick, 1966–A mathematical study of the kinetics of biological oxidation. M.S.

626. Tomme, Michael Henry, 1974–Algal bioassays applied to Central Texas waters. M.S.

627. Tribbey, Bert Allen, 1965–A field and laboratory study of ecological succession in temporary ponds. Ph.D.

628. Trevino, D.B., 1955–The ichthyofauna of the lower Rio Grande River, from the mouth of the Pecos to the Gulf of Mexico. M.S.

629. Tu, Catharina Tse-Ying Yang, 1971–The study of a phosphate-containing fluorescent compound from spinach chloroplasts. M.S.

630. Uhlik, David James, 1969–Studies in *Chlamydomonas*. M.A.

631. Urban, L.V., 1966–Estimates of physical exchange in Galveston Bay, Texas. M.S.

632. ————, 1971–A laboratory investigation of temperature and velocity fields in a stratified cooling reservoir. Ph.D.

633. Valencia-Montoya, Guillermo, 1970–Oxygen consumption by benthic deposits. M.S.

634. Van Dover, Byron, 1972–Studies of the ultrastructure and reproduction of several species of *Chlamydomonas* and *Cateria*. Ph.D.

635. Volkmann, Carol Mary, 1960–The microbial decomposition of organic carbon in surface sediments. M.S.

636. Voswinkel, Linda Louise, 1969–Taxonomy and ultrastructure of the algal genus *Pyrobotrys*. M.S.

637. Wadsworth, Albert Hodges, 1941–The lower Colorado River, Texas. M.A.

638. Wallace, James William, Jr., 1967–Investigations of flavone biosynthesis in the Lemnaceae. Ph.D.

639. Walz, David Henry, 1974–Sewage renovation and surface-water quality, Lakeway Resort Community, Travis County, Texas. M.S.

640. Watkins, G.M., 1930–Vegetation of San Marcos Springs. M.S.

641. Watson, Melvin W., 1970–Ultrastructural aspects of development in *Microthamnion*. Ph.D.

642. Weber, Gerald Eric, 1968–Geology of the fluvial deposits of the Colorado River Valley, Central Texas. M.S.

643. Wells, Dan Moody, 1966–Management of return flows in Texas. Ph.D.

644. Wenzel, Roger Allen, 1975–Effects of floods on selected sand bars, San Bernard and Brazos Rivers, Austin County, Texas. M.S.

645. White, Dale Wesley, 1974–Survey of pollution characteristics of intensely cultured fish. M.S.

646. Wiedeman, Varley Earl, 1964–Some aspects of algal ecology in a waste-stabilization pond system. Ph.D.

647. Wiland, Bruce Lane, 1975–Transient state biological kinetics. M.S.

648. Wilkins, Lowell H., 1973–Wave spectra and associated statistical characteristics of free-surface waves on a freshwater lake. Ph.D.

649. Williams, George Jackson, III, 1969–Comparative phenology, physiology, and biochemistry of populations of *Liquidambar styraciflua* L. Ph.D.

650. Williams, John Gregor, 1974–A comparison of development rates and temperature tolerances of two species of Cyprinodont fishes (Family Cyprinodontidae): *Cyprinodon variegatus* and *Cyprinodon bovinus*. M.S.

651. Wilson, Joe Robert, 1967–Field investigation of mixing and dispersion in a deep reservoir. Ph.D.

652. Woodruff, Charles Marsh, Jr., 1973–Land-use limitations related to geology in the Lake Travis vicinity, Travis and Burnet Counties, Texas. Ph.D.

653. Wu, Madeline Chang Sun Wang, 1966–Pentose inhibition of the growth of *Chlorococcum echinozygotum*. Ph.D.

654. Yang, Julie Tsu-yu, 1968–A study of selected isoenzymes in Lemnaceae. M.S.

655. Yantis, Hugh Cleveland, Jr., 1965–Water quality criteria for Brushy Creek in Williamson County, Texas. M.S.

656. Yardley, Darrell Gene, 1972–An ecological and electrophoretic analysis of the interaction between *Gambusia affinis* and *G. heterochir*. M.S.

657. Yarish, Charles, 1972–Experimental taxonomic studies of certain marine chaetophoracean algae. M.A.

658. Young, W.C., 1960–Ecological studies of commensal Branchiobdellidae (Oligochaeta) and Entocytheridae (Ostracods) on *Procambarus simulans simulans* (FAXON) (Decapoda). Ph.D.

659. Young, Donald Ray, 1972–The relationship of land use to water use in San Antonio, Texas. M.S.

660. Young, Frederick LeRoy, Jr., 1965–Vacuum filtration of algae. M.S.

661. Young, Joncie Harvey, 1974–Facultative waste stabilization pond processes, performance, design, and predictive models. M.S.

662. Yousef, Ariz Yousef, 1962–Coliform in waste stabilization ponds. M.S.

UNIVERSITY OF TEXAS AT DALLAS

663. Gary, M., 1976–Factors controlling the eutrophication of Lake Ray Hubbard. Ph.D. (In progress)

664. Meyers, M., 1976–An evaluation of the factors influencing water quality of White Rock Lake. M.S. (In progress)

665. Rast, Walter, 1976–An evaluation of the relationship between aquatic plant nutrient loads and the degree of fertilization of lakes and impoundments. Ph.D. (In progress)

UNIVERSITY OF TEXAS AT EL PASO

666. Ferguson, J.A., 1961–Water for international rivers; the Rio Grande as a model. M.S.

667. Haney, P.L., 1948–The international controversy over the waters of the Rio Grande. M.A.

668. Kirby, J.W., 1968–Water resources, El Paso County, Texas: past, present, future. M.A.

669. Nicoll, M.C., 1951–Brief history of the El Paso water system from 1881-1921. M.A.

670. Sedillo, F.J., 1969–Investigations on the recharge to groundwater by the sewage effluent of WSMR, New Mexico. M.S.

671. Stachowiak, A.S., 1969–Groundwater resources of the river alluvium. M.S.

672. White, Alice M., 1950–History of the development of irrigation in El Paso Valley. M.A.

UNIVERSITY OF HOUSTON

673. Powell, R.W., 1953–Diatoms of Bray's Bayou, Houston, Texas. M.S.

WEST TEXAS STATE UNIVERSITY

674. Anderson, G., 1975–Bacterial quality of Lake Meredith. M.S. (In progress)

675. Canada, W.B., 1975–Water quality of Lake MacKenzie, M.S. (In progress)

676. King, E.D., 1969–Coliform bacteria in Lake Meredith, Texas. M.S.

677. Hestand, R.S., III, 1966–Quality of water in Lake Meredith, Texas. M.S.

678. Haralson, J.E., 1972–The problem of providing a dependable quality municipal water supply for the City of Winfield, Kansas. M.S.

679. Jones, O.R., 1970–The effect of net plankton on the recharge rate of wells and spreading basins. M.S.

680. Lutz, J.D., 1970–Age, growth, and condition of Northern Pike (*Esox lucius*) in Greenbelt Lake in the Texas Panhandle. M.S.

681. Meriage, M., 1975–Checklist on amphibians and reptiles of Randall County. M.S. (In progress)

682. Newton, C.E., 1968–Phytoplankton succession and seasonal periodicity in a maturing lake. M.S.

683. Whitney, W.E., 1967–Canadian River dam project in Texas. M.S.

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ANGELO STATE UNIVERSITY

684. Fischer, Lyndal, 1976–The Caddisflies of the Concho River System. M.S. (In progress)

BAYLOR UNIVERSITY

685. Jacobs, Robert, 1976–The combined relationship of temperature and molybdenum concentration to nitrogen fixation by *Anabaena cylindrica*. M.S.

686. Neely, Robert, 1976–The contribution of aquatic macrophytes to primary productivity in two Texas reservoirs. M.S. (In progress)

687. Short, James, 1976–Effects of dissolved organics on nutrient availability. M.S. (In progress)

688. Muschler, Lee, 1976–The phosphorus budget of two Texas reservoirs. M.S. (In progress)

LAMAR UNIVERSITY

689. Griffith, Wliiam H., 1975–Acute toxicity of copper and lead to the blue crab, *Callinectes sapidus* Rathbun. M.S.

690. Linney, George K., 1975–Minimum temperature tolerance of *Rhizophora mangle* saplings in culture. M.S.

SAM HOUSTON STATE UNIVERSITY

691. Hand, S.S., 1976–A comparison of three methods of establishing primary productivity of surface water of Lake Conroe. M.S.

PAN AMERICAN UNIVERSITY

692. Atkinson, Eduardo, 1976–Comparative hematology of the Rio Grande cichlid, *Cichlasoma cyanoguttatum*, and the redear sunfish, *Lepomis microlophus*. M.S.

STEPHEN F. AUSTIN UNIVERSITY

693. Allen, Charles E., 1976–Bioeconomics and feeding habits of ducks in flooded bottomlands of Eastern Texas. M.S.

694. Lindsey, Larry R., 1976–An analysis of coliform and streptococcus bacteria as indicators of fecal pollution in the Attoyac River. M.S.

695. Richardson, Larry J., 1976–Analysis of the coliform and streptococcus bacteria as indicators of fecal pollution in the Angelina River. M.S.

TEXAS A&M UNIVERSITY

696. Grosmaire, Eric G., 1976–Food analysis and population dynamics of fresh water turtles in the Rio Grande Valley of south Texas. M.S.

697. Hays, Bill, 1976–The comparative ethology of various genera of Texas crayfish. Ph.D.

UNIVERSITY OF HOUSTON

698. Carr, Barbara Ann, 1972–Assumption of L-valine and D-glucose by the mid-gut gland of the shrimp, *Penaeus aztecus*. M.S.

699. Castille, Frank, 1976–The uptake of hexose from seawater by post-larval Penaeid shrimp. M.S.

UNIVERSITY OF TEXAS AT DALLAS

700. Foute, Steve, 1976–Land usage patterns within the Lake Ray Hubbard, Texas, watershed. Ph.D. (In progress)

701. Meckel, Elaine, 1976–Biological responses of Lake Ray Hubbard, Texas, to phosphorus and nitrogen loadings. M.S. (In progress)

702. Moore, Mike, 1976–Sedimentation patterns as they relate to the phosphorus and nitrogen budgets of Lake Ray Hubbard, Texas. M.S. (In progress)

703. Rahman, Magda, 1976–Development of a eutrophication model for Lake Ray Hubbard, Texas. Ph.D. (In progress)

THE UNIVERSITY OF TEXAS

SCHOOL OF PUBLIC HEALTH AT HOUSTON

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704. Abd-Eldayen, Mohamed Sabry, 1973–A feasibility study of the non-chlorination methods for potable water disinfection - - (with reference to proposed disinfection of the Holy Water of Zamzam well in Mecca). M.P.H.

705. Baird, Justus N., 1973–Salmonella, turtles, and public health. M.P.H.

706. Bean, Judy A., 1973–Behavior of replication and linearization variance estimators for complex multistage probability samples. Ph.D.

707. Casserly, Dennis M., 1976–Indicator and pathogenic bacteria dynamics of urban and rural stormwater runoff. M.S.

708. DiPietro, Walter P., 1975–Bacteriological investigation of private well water supplies in Acres Homes (Houston). M.S.

709. Dunn, William F., 1971–Nutrient Flux in the San Antonio Bay complex as measured by water column analysis. M.S.

710. Fosbury, Walter Jay, 1972–The use of aerial remote sensing for the detection of specific water pollution parameters. M.S.

711. Gomez, Sabino, 1971–The significance of viruses in water. M.P.H.

712. Gothard, Clair S., 1974–Bacteriological analysis of surface water by the technique of a continuous monitoring system. M.P.H.

713. Gray, William G., 1973–Ammonia levels in Texas streams receiving municipal wastewater effluents during periods of low flow. M.P.H.

714. Hall, Robert C., 1970–Study of sanitary land fills as a potential health hazard. M.P.H.

715. Henderson, Marilyn, 1976–Some factors affecting the enumeration of viruses isolated from water and wastewater. M.S.

716. Hulka, Steven C., 1972–The occurrence of indicator organisms in two Texas estuaries. M.S.

717. Jackson, Gaines B., 1972–A treatise on the community health aspects of water quality in Chocolate Bayou. M.S.

718. Keen, Spruce R., 1972–Postchlorination bacterial survival and aftergrowth in secondary treated wastewater. M.S.

719. Langdon, William C., 1972–Primary productivity and nutrient budget of San Antonio Bay - a Texas estuary. M.S.

720. Moore, James D., 1975–Multiple regression analysis of bacterial populations in a natural stream. M.S.

721. Reeve, Gordon R., 1975–Cholinesterase levels of aerial applicators exposed to organophosphate pesticides. M.P.H.

722. Simpson, Diane M., 1974–Bacteria in treated domestic sewage used for irrigation: identification of the enterobacteriaceae emitted from a sprinkler and observations of the coliform concentrations present within the system. M.P.H.

723. Smith, George Franklin, 1973–A preliminary investigation into the nutrient requirements of four Texas estuarine systems. M.S.

724. Smith, Billy R., 1972–A comparison of two bacteriological analysis techniques in brackish waters. M.P.H.

725. Tapp, Mark, 1975–An evaluation of the eutrophication rate and some health related factors in the Clear Lake Basin (Texas). M.S.

726. Valentine, Jane L., 1973–Distribution of trace elements in water in the Houston environment: relationship to mortality from arteriosclerosis, heart disease. Ph.D.

727. Weese, Thomas H., 1973–The study of the possible effects of salinity, water temperature and hydrological conditions on *Penaeas setiferus* (Linnaeus). M.S.

728. Yates, Matthew M., 1974–Field and laboratory observations of possible interactions between some acquatic algae and *Culex pipiens quniquefasciatus* (The Southern House mosquito). M.S.

NORTH TEXAS STATE UNIVERSITY

729. Atmar, Gerald L., 1970–Food, feeding and ecological efficiencies of *Fundulus notatus* (Rafinesque) (Osteichthyes; Cyprinodontidae) Ph.D.

730. Bokich, J.M., 1976–Demography and life history energetics of White Bass, *Morone chrysops*. Ph.D. (In progress)

731. Burleson, J., 1976–Effect of chlorine on amino acids and polypeptides in municipal wastewaters. M.S. (In progress)

732. Childress, M., 1976–Systems model of energy flow and trophic dynamics of a North Central Texas pond ecosystem. M.S. (In progress)

733. Dermanci, Alki, 1976–Size distribution and chlorine contents of treated wastewater effluents. M.S. (In progress)

734. Fagan, J., 1976–Life history energy allocation to growth and reproduction in Gizzard Shad. M.S.

735. Fullington, Richard W., 1976–The aquatic gastropods of Texas. Ph.D. (In progress)

736. Fullington, Kate E., 1976–Nymphs of the stonefly genera, *Taeniopteryx* and *Zealeuctra* of North America. M.S. (In progress)

737. Gash, Stephen L., 1971–Effects of Carbaryl (1-napthyl-N-Methylcarbamate) on *Trichocorixa reticulata* (Hemiptera:Corixidae) and *Glyptotendipes barbipes* (Diptera : Chironomidae). Ph.D.

738. Grant, Peter, 1976–Life cycle of the mayfly *Isonychia sicca manca* (Eaton) in Clear Creek and the Brazos River, Texas. M.S. (In progress)

739. Glidewell, J., 1976–Life history, population and niche analyses of Red Slider turtles in North Central Texas. Ph.D. (In progress)

740. Huang, Francis, 1976–Photolytical oxidation of polynuclear aromatic hydrocarbons in aqueous solutions. M.S. (In progress)

741. Henderson, James E., IV, 1976–GC/MS studies of organochlorine compounds formed by the chlorination of municipal wastewaters. Ph.D. (In progress)

742. James, Gary L., 1976–Genetic variation in red shiners, *Notropis lutrensis* in the Brazos and Trinity Rivers. M.S. (In progress)

743. Jones, J., 1976–Biochemical variation in the top minnow *Fundulus*. M.S. (In progress)

744. Lin, Simon, 1976–Photolytic oxidation of trihalomethanes in aqueous solutions. M.S. (In progress)

745. McCullough, W., 1976–Phytoplankton-salinity relationships in Lake Texoma. M.S. (In progress)

746. Merritt, R., 1976–Genetic variation and the effects of isolation on the stoneroller, *Campostoma anomalum*. M.S. (In progress)

747. McClure, Richard G., 1974–The life history and ecology of the mayfly *Neochoroterpes mexicanus* Allen (Ephemeroptera:Leptophlebiidae) M.S.

748. Perry, W.B., 1976–Biomass and carbon relationships in water and sediment of a mesotrophic Texas reservoir. M.S. (In progress)

749. Oberndorfer, Reed Y., 1976–The life cycle of *Hydroperla crosbyi* (Needham Claassen) (Plecoptera:Perlodidae) M.S.

750. Poole, Walton C., 1973–The vertical stratification of the macrobenthos in the Brazos River, Texas. M.S.

751. Richmond, M.C., 1976–Evolutionary genetics of red shiners, *Notropsis lutrensis*, inhabiting a cooled effluent from a reservoir. M.S. (In progress)

752. Shelton, Bonnie K., 1976–The use of scanning electron microscopy in Limnology. M.S. (In progress)

753. Snellen, Rosalyn K., 1976–Life cycle of *Zealeuctra claasseni*, *Zealeuctra lutei*, *Perlesta placida* (Plecoptera) and *Chimarrha obscura* (Trichoptera) in North Texas. Ph.D. (In progress)

754. Widner, D., 1976–Stream ecology of White Bass *Morone chrysops* in North Central Texas. M.S. (In progress)

755. Ziegler, David D., 1976–Drumming behavior of selected Nearctic Stoneflies (Plecoptera). M.S.

UNIVERSITY OF TEXAS AT AUSTIN

756. French, Dorian, 1976–Heavy metal analysis of raw water supply and wastewater discharge for the city of Austin, Texas. M.S.

756a Hoffman, H.W., Jr., 1976–A continuous equation to describe bioassay results for acute exposure of fish to toxins. M.S.

757. Neuse, David W., 1976–The removal of algae from an oxidation pond effluent through the use of a tertiary water hyacinth pond system. M.S.

758. Roques, Pat F., 1975–Effects of fluctuating nutrient and regimes on autotrophic succession in microecosystem. M.A.

TEXAS CHRISTIAN UNIVERSITY

759. DeLuca, Robert C., 1976–A study of the interrelationship of naturally collected bacteria and several species of algae. M.S.

760. O'Kane, Kevin D., 1976–A population study of the exotic bivalve *Corbicula manilensis* (Philippi, 1841) in selected Texas reservoirs. M.S.

TEXAS A&M UNIVERSITY

761. Camejo, Daniel, 1941–Typical anopheles breeding places formed by irrigation. M.S.

762. Crocker, B.R., 1975–An inventory of the original vegetation of the Palmetto Bend reservoir site in Jackson County, Texas with respect to potential eutrophication. M.S.

763. Coffey, J.E., 1956–Flood in the Nueces, Guadalupe, Lavaca and Mission River Basins: magnitude and frequency. M.S.

764. Hayes, W.A., 1976–Comparative studies of agonistic behavior in cambarid crayfish. Ph.D.

765. Holt, G.J., 1976–Community structure of macrozooplankton in Trinity and upper Galveston Bays, with special reference to the cooling water system of Cedar Bayou Electric Generating Station. Ph.D.

766. Hall, E.R., 1976–Water quality of some coastal canal communities in the Galveston County area. M.S.

767. Janssesn, H.E., 1976–Distribution and fate of technical Chlordane and Mirex residues in a central Texas aquatic ecosystem. M.S.

768. Langford, T.R., 1948–A study of transition of flow in open channels with flow at a sub-critical depth. M.S.

769. Maldonado, R.J., 1976–Steady state estuarine modeling of the Brownsville Ship Channel. M.S.

770. Priebe, W.F., 1972–Oxygen uptake of bentic systems. M.S.

771. Rahman, K., 1963–Study of the effect of rate of sediment transport on the stability of open channels. M.S.

772. Reavis, M.W., 1976–The environmental management of a ship channel complex. M.S.

773. Smith, R.H., 1968–Survey of organic content in bottom of the Houston Ship Channel. M.S.

774. Vigil, S.A., 1974–Nutrient sources and loading for the proposed Millican Lake. M.S.

775. Withers, R.E., 1969–Salinity patterns in the Houston Ship Channel. M.S.

776. Walsh, Don, 1968–The Mississippi River outflow, its seasonal variations and its surface characteristics. Ph.D.

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1, 4, 5, 7, 8, 9, 16, 17, 18, 19, 22, 23, 24, 27, 32, 61, 67, 68, 70, 87, 88, 91, 93, 94, 96, 103, 106, 107, 108, 110, 111, 124, 125, 127, 138, 143, 148, 149, 150, 151, 154, 158, 166, 172, 184, 199, 203, 206, 209, 211, 214b, 214e, 219, 221, 224, 227, 231, 235, 239,2 243, 254, 265, 269, 270, 274, 279, 281, 283, 286, 287, 288, 288b, 290, 291, 292a, 294, 296, 301, 302, 309, 314, 315, 316, 319, 320, 323, 325, 329, 331, 335, 336, 337, 341, 343, 345, 346, 347, 353, 359, 362, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 422, 423, 426, 427, 428, 435, 436, 437, 439, 441, 446, 449, 474, 480, 493, 496, 497, 518, 524, 525, 527, 537, 540, 544, 560, 561, 564, 599, 613, 618, 624, 628, 645, 650, 656, 680, 729, 730, 734, 742, 743, 751, 754, 756a

*Macrophytes and Periphyton*
11, 31, 74, 100, 192, 200, 207, 240, 300, 321, 328, 376, 378, 434, 482, 457, 459, 491, 570, 607, 638, 640, 649, 654, 665, 686, 690, 757

*Management, General Hydrology, History, Law, Economics, Education*
13, 146, 202, 204, 205, 207, 251, 259, 272, 278, 285, 290, 304, 322, 369, 370, 371, 373, 425, 475, 476, 479, 484, 464, 465, 466, 495, 517, 523, 532, 538, 586, 588, 637, 644, 652, 655, 659, 666, 667, 668, 669, 672, 678, 683, 700, 702, 763, 768, 771, 772, 773, 775

*Microbiology, Bacteriology, Mycology*
15, 46, 48, 57, 62, 63, 65, 71, 73, 74, 79, 84, 86, 92, 102, 104, 132, 161, 216, 228, 271, 307, 520, 584, 593, 635, 662, 674, 676, 694, 695, 704, 705, 707, 708, 711, 712, 714, 715, 716, 718, 720, 722, 724, 752, 759,

*Modeling, Biological Indexes*
122, 222, 292a, 465, 471, 495, 512, 526, 535, 538, 552, 554, 567, 572, 588, 595, 596, 625, 647, 700, 703, 706, 720, 732, 734, 756a, 769, 775, 776

*Mollusca*
40, 147, 159, 162, 167, 168, 174, 175, 181, 203, 218, 252, 253, 260, 293, 330, 342, 355, 429, 735, 760

*Nutrients, Eutrophication, Water Quality*
3, 20, 54, 76, 80, 83, 98, 113, 123, 125, 126, 139, 141, 170, 183, 210, 214i, 214k, 215, 229, 238, 241, 245, 257, 263, 264, 276, 289, 290, 292, 294, 303, 306, 308, 310, 315, 348, 351, 360, 363, 365, 370, 374, 438, 439, 442, 453, 478, 463, 465, 468, 491, 502, 511, 521, 530, 532, 547, 549, 550, 577, 581, 591, 598, 600, 601, 609, 629, 635, 639, 655, 663, 664, 665, 675, 677, 685, 687, 688, 694, 695, 700, 701, 702, 703, 709, 710, 713, 714, 717, 719, 723, 725, 748, 758, 762, 766, 774

*Physico-chemical, Geochemistry, Water Chemistry, Tastes and Odors*
12, 14, 33, 36, 37, 38, 40, 41, 44, 45, 57, 65, 81, 82, 84, 85, 88, 93, 117, 118, 123, 128, 151, 157, 183, 191, 204, 208, 211, 213, 214c, 214g, 214k, 223, 226, 229, 230, 232, 241, 276, 284, 289, 303, 304, 305, 306, 310, 318, 348, 351, 357, 360, 438, 439, 451, 476, 485, 486, 494, 519, 553, 567, 568, 591, 594, 599, 606, 631, 632, 633, 639, 648, 651, 655, 664, 745, 770, 775, 776

*Phytoplankton* (Algae)
6, 20, 28, 39, 49, 50, 51, 52, 55, 59, 69, 72, 77, 78, 81, 85, 98, 99, 100, 102, 109, 115, 116, 118, 119, 121, 125, 130, 133, 136, 139, 142, 144, 153, 155, 157, 163, 164, 173, 180, 195, 212, 214a, 236, 244, 246, 250, 258, 264, 268, 289, 295, 298, 300, 307, 321, 334, 340, 358, 372, 432, 434, 444, 445, 447, 448, 452, 453, 454, 478, 483, 458, 460, 462, 467, 470, 472, 489, 490, 492, 498, 501, 504, 505, 506, 507, 511, 516, 522, 531, 536, 539, 541, 542, 543, 546, 548, 555, 556, 562, 563, 565, 569, 573, 574, 575, 576, 577, 582, 589, 590, 597, 602, 604, 605, 611, 612, 614, 617, 619, 620, 622, 623, 629, 630, 634, 636, 641, 646, 653, 657, 660, 673, 679, 682, 685, 701, 728, 745, 757, 758, 759

*Primary Production* (Chlorophyll a, Biomass, Photosynthesis)
54, 95, 126, 133, 135, 140, 163, 186, 214g, 214k, 236, 244, 246, 282, 295, 300, 324, 334, 483, 500, 506, 534, 539, 686, 691, 719

*Radioactive*
499, 509, 516, 582, 598, 608

*Sewage, Effluents, Treatment* (Oxidation ponds)
35, 36, 43, 80, 92, 146, 148, 163, 194, 280, 312, 344, 365, 442, 443, 481, 488, 502, 510, 515, 528, 564, 571, 581, 598, 615, 621, 639, 645, 661, 670, 713, 715, 718, 722, 731, 733, 741, 756, 757

*Thermal, Electric Power*
11, 17, 20, 21, 64, 96, 100, 107, 111, 123, 133, 138, 147, 157, 187, 214a, 214k, 233, 236, 279, 281, 283, 286, 287, 288a, 288b, 291, 292a, 299, 301, 309, 314, 314a, 317, 323, 325, 333a, 335, 336, 337, 339, 358, 373, 431, 433, 464, 500, 513, 524, 537, 559, 605, 613, 614, 621, 631, 632, 650, 685, 689, 727, 751, 765

*Urban Runoff, Storm Runoff, Non-point Source*
35, 114, 194, 206, 209, 211, 247, 266, 297, 363, 366, 367, 368, 377, 440, 477, 508, 579, 596, 603, 610, 707

*Zooplankton*
39, 47, 99, 109, 115, 119, 129, 130, 145, 166, 179, 182, 190, 212, 214f, 214j, 217, 234, 249, 275, 288a, 317, 326, 338, 340, 354, 358, 431, 456, 550, 559, 566, 616, 765

# IS CANOPY INTERCEPTION AN ACCURATE MEASURE OF LOSS FROM THE HYDROLOGIC BUDGET?

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## INTRODUCTION

Interception is the process by which the aerial portions of vegetation intercept and retain precipitation until it is evaporated. The amount of precipitation that is intercepted by vegetation, estimated at 10-20% of the annual total (Linsley, *et al.,* 1975), varies with storm intensity and duration, precipitation type, wind speed, type of plant cover, and season of the year. Since intercepted water does not reach the ground and contribute to soil moisture or streamflow, it is widely considered as a loss to the hydrologic budget.

The evaporation of intercepted water is similar to water loss from an open water surface. Due to the small diffusive resistance at the evaporating surface, it is expected that evaporation of intercepted water occurs at a greater rate than transpiration under similar atmospheric conditions. During evaporation of intercepted water, the energy available for transpiration is reduced, and a reduction in transpiration is expected. Interception therefore should not be a complete loss from hydrologic budgets. This idea has been discussed by Christiansen (1942), Goodell (1963), Penman (1963), Lull (1966), and others, and studied by Rakhmanov (1958), Burgy and Pomeroy (1958), McMillan and Burgy (1960), and more recently by Harr (1966), Thorud (1967), and Nicolson, *et al.,* (1968).

Investigators agree that evaporation and transpiration are energy dependent processes, but appear to differ on the availability of energy for them. One group feels that evaporation of intercepted water preempts energy which would have been used for transpiration. Others appear to believe that more energy is available for evaporation over a wet canopy as compared to a dry canopy. Thus, Murphy and Knoerr (1975) state that "interception of precipitation does represent a loss of water to the soil and streamflow under field conditions." It is the purpose of this paper to evaluate the question: "Should canopy interception be considered as a loss in the hydrologic budget, or not?"

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## INTERCEPTION MEASUREMENTS

The interception process is composed of 2 phases: 1) interception storage, or retaining precipitation in the vegetative canopy, and 2) evaporation of the intercepted water. Interception loss, properly defined, is the amount of evaporation minus the amount of transpiration reduction. Since direct simultaneous measurement of transpiration and interception loss is not possible, estimates must be based on indirect methods.

Usually, interception is estimated by comparing precipitation measured in the open, $P_O$, and that under the vegetative canopy, $P_C$, with a correction for stemflow, i.e., for the water which runs down the vegetative stems, $P_S$. Thus the canopy interception, $I_C$, can be calculated by the following equation

$$I_C = (P_O - P_C) - P_S \quad (1)$$

$P_O$ and $P_C$ in Equation 1 are also refered to as gross precipitation and net precipitation respectively. Water intercepted by forest litter, a related phenomenon requiring other measurement approaches, is not included in this discussion.

The concept of Equation 1 has been widely employed in scientific research and for practical purposes by Beall (1934), Rowe and Hendrix (1951), Semago and Nash (1962), Leonard (1961), Miller (1964), Rogerson and Byrnes (1968), and many others. Stemflow ($P_S$) can be measured by a rolled gutter attached to the tree trunk, the catch from which is drained into a container. The volume of water collected in containers is measured and converted into depth over the sampled areas. Precipitation in openings (gross precipitation, $P_O$) and under vegetative canopies (throughfall, $P_C$) is measured with multiple conventional rain gages, under careful sampling techniques (Helvey and Patric, 1965; Lawson, 1967; Zinke, 1967).

## INTERCEPTION STUDIES

### *New Findings*

Rakhamanov (1958) observed the transpiration rates of sprinkled and unsprinkled excised plant branches, whose bases were sealed into a water filled bottle. Water loss due to transpiration was determined by weighing, and expressed as the quantity of water transpired per unit fresh weight of the branch. He found that transpiration of aspen, downy birch, hazels, yellow acacia, oak, and sedge basket willow was reduced from 15 to 40% by spraying. The author concluded: "The precipitation intercepted by the tree crowns . . . cannot be considered as a loss to the water balance of the forest soils."

Burgy and Pomeroy (1958) studied interception by grassy species grown in nutrient solutions in a greenhouse. Rates of evapotranspiration from sprayed and unsprayed pans were compared. "The evaporation of a given amount of intercepted moisture," the authors found, "was accompanied by a like reduction in the amount of evapotranspiration from the plants." The total moisture loss in

sprayed and unsprayed pans was about the same. Thus, the authors suggested that the terminology of interception loss should be re-examined on the basis of the net effect.

Later McMillan and Burgy (1960) measured evapotranspiration from sprayed and unsprayed vigorous grass covers in floating lysimeters in a grass field. They concluded that, on the average, the difference between sprayed and unsprayed plots was not significant, a result consistent with that of the earlier greenhouse study. Lee (1968), however, called attention to the fact that, during the first hours after spraying, evapotranspiration increased by 16%.

Thorud (1967) evaluated interception-transpiration relationships of small trees. Treatment and control groups were randomly selected from ten 6- to 7-yr-old ponderosa pines well established in 10-qt plastic pails. The pail, and the base of each tree, were sealed with plastic to prevent evaporation from the soil, and transpiration was determined by weight differences. Before applying water to the trees, the relationship of transpiration rates between control and treatment groups were calibrated. Water of known quantities was then sprayed on the treatment trees and the concurrent transpiration losses of control and treatment trees were measured simultaneously. The amount of transpiration reduction was estimated with an equation based on the calibration data. The results showed that about 9% of the applied water was conserved in the soil. In other words, about 91% of the applied water was a net interception loss to soil moisture. The author concluded that transpiration reduction was small compared with the losses implied by earlier studies of interception by grass.

In similar studies with Colorado blue spruce and Austrian pine in small pots, Harr (1966) found average reductions in transpiration of about 6%. Nicolson, *et al.*, (1968), using white spruce and white pine in Minnesota, measured average savings as about 12% of the intercepted water applied.

The studies cited above provided evidence that intercepted water reduced transpiration and consequently conserved soil moisture. The amount of water conserved depended in part on weather conditions (Nicolson, *et al.*, 1968), and varied among species.

*Energy Source*

Since evaporation is an energy dependent process, many have attempted to interpret interception loss in terms of available energy. For example, in discussing the special difficulties of estimating evaporation, Penman (1963) states:

> . . . while the leaves are wet the evaporation is effectively as from an open water surface rougher (sic) than a normal plane surface so that the evaporation rate will be greater during the day than that of transpiration of soil water, and can go on at night. But the same energy cannot be used twice, and while the intercepted water is being evaporated the drain on soil water is checked. Intercepted water is not wasted; though its use may be slightly luxurious.

Penman's (1963) statement on the evaporation rate from a wet canopy is supported by several studies. In a stand of Scots pine in southeast England, Rutter (1967) found that intercepted water evaporated, on average, about 4 times as fast as the transpiration rate under the same environmental conditions. Murphy and Knoerr's (1975) simulation study indicates that evaporation of wet leaves may be from 1.5 to more than 2 times greater than that of dry leaves. They further explain that the energy source for the greater evaporation of a wet canopy comes from an increase in net radiation through "a decreased long-wave radiation and a decreased or even negative sensible heat flux." Thus, they do not agree on the hypothesis that the amount of energy available for evaporation is fixed in a particular environment, and claim that precipitation interception does represent a hydrologic loss under field conditions. Apparently, they ignore the 10% compensating reduction in evapotranspiration caused by the evaporation of intercepted water.

## EVALUATION

These studies agree that evaporation of intercepted water is greater than the transpiration from dry leaves under the same environmental conditions. The interpretation of intercepted water in the hydrologic budget, however, remains controversial. This report will consider the interception process from three viewpoints: available energy, water vapor diffusive resistance, and plant physiology.

### *Available Energy*

The energy required to change water from liquid to vapor at ordinary air temperature is about 580 cal/g. The energy for sustained vaporization of water from a vegetative surface, such as a forest is primarily from solar radiation. The net radiation, S, of a stand is given by

$$S = (I + H)(1 - r) + G - \sigma T^4 \qquad (2)$$

where I is direct solar radiation, H is sky radiation, r is the reflectivity of incoming short-wave radiation or so-called albedo, G is long-wave radiation from the atmosphere, and $\sigma T^4$ is the long-wave radiation emitted by the forest, in which T is surface absolute temperature in °K, and $\sigma$ is Stephen-Boltzmann constant = $8.26\ (10^{-11})$ cal/cm$^2$min°K$^4$. The albedo, r, is affected by the nature of a surface, and to some extent by its moisture content. For example, Leyton, *et al.*, (1967) found that wetting sharply reduced the albedo of a spruce canopy in England and the albedo gradually increased as the foliage dried out.

From Equation 2 it is evident that albedo, r, and surface temperature, T, are the only 2 surface parameters that can change the net radiation. Albedo, on average, is about 0.14 for coniferous forests, and up to 0.25 for deciduous trees; it is about 0.05-0.07 for a free water surface (Bruce and Clark, 1966) at high sun angles. A canopy wetted by intercepted water probably has a lower albedo than a dry canopy. On the other hand, the canopy temperature is lowered when intercepted

water is evaporated. Rutter (1967) found that the wet leaves of Scots pines were 1.0 C cooler than the air. Consequenctly, with a lowered canopy temperature, the canopy outgoing radiation, $\sigma T^4$, is reduced. Equation 2 shows that negative changes in r and $\sigma T^4$ caused by the presence of intercepted water will cause a positive change in S.

The net energy delivered to a forest stand is dissipated primarily by convection, H, vaporization, L, and conduction, B, as indicated by the equation:

$$S = H + L + B \tag{3}$$

From Equation 3, the heat flow of vaporization is given by:

$$L = S - (H + B) \tag{4}$$

Equation 4 indicates that vaporization increases with S and decreases with H + B. But B is usually a small portion of the total available energy in a forest. Therefore, L is mainly affected by the available energy, S, and the heat exchange between the canopy and the atmosphere, H. According to the above analysis, a wet canopy would cause a positive change in S, and, because of a reduction of surface temperature, the sensible heat flux, H, is reduced or even negative. This will result in a higher rate of vaporization, L.

*Diffusive Resistance*

That the evaporation of intercepted water is faster than the transpiration from the leaf can be explained in terms of relative resistances in the water vapor diffusion pathways. Consider the following equation,

$$T\ (g/cm^2\,sec) = (C_s - C_a)/(R_f + R_a) \tag{5}$$

where T is the transpiration rate, $C_s$ and $C_a$ are the water vapor concentrations ($g/cm^3$) at the evaporating surface within a leaf and in the bulk air respectively, and $R_f$ and $R_a$ are the diffusive resistances (sec/cm) in the leaf stomates and in the surface boundary layer of air outside the leaf. Equation 5 is mainly a function of temperature if diffusive resistance remains constant, because $C_s$ is uniquely determined by temperature. However, if weather conditions remain constant, the diffusive resistance will play an important role in determining the transpiration rate. When water is intercepted at the leaf surface, $R_f = 0$, and the total resistance is reduced, and Equation 5 becomes

$$E\ (g/cm^2\,sec) = (C_s - C_a)/R_a \tag{6}$$

where E is the evaporation rate of intercepted water. Rutter (1967) in his Scots pine study found that the resistance to water vapor diffusion from a wet leaf was 1.24 sec/cm, while that for transpiring leaves with dry surfaces was 9.1 sec/cm. The large difference in diffusive resistances explains the higher evaporation rate from wet leaves.

*Plant Physiology*

Physiologically, water movement through the soil-plant-atmosphere system is mainly controlled by water potential gradients in the soil and plants, and by the vapor pressure gradients between evaporating surfaces and the air (Kramer, 1969). As water is transpired from leaves, the water potential in the leaf cells is reduced. This causes water to move from the area of higher water potential in the xylem of the leaf veins into leaf cells. Removal of water from the xylem reduces its water potential, and this reduction is transmitted through the water conducting systems of the plant and into the soil. The energy required for these water movements, together with the fact that transpiration from leaves is largely limited to interior surfaces of stomatal openings, account for the greater diffusive resistance to transpiration.

Rutter's (1967) study demonstrated that "the rate of evaporation of intercepted water is, on average, about 4 times as great as the transpiration rate in the same environmental conditions." Intercepted water, presenting an open surface to the air is obviously more available to evaporation than the water in leaf cells, and must be evaporated before transpiration can occur. Since one source of energy cannot be used in two different places, energy dissipated by evaporating the intercepted water at the leaf surface reduces the energy available for leaf transpiration. It should be noted, however, that the energy available for evaporating the intercepted water is not necessarily a fixed fraction of solar radiation. The reduction in transpiration caused by intercepted water, ranging from 100% (Burgy and Pomeroy, 1958; McMillan and Burgy, 1960) to about 10% (Harr, 1966; Thorud, 1967; Nicolson, *et al.*, 1968), depends on the duration of intercepted water on the leaf's surfaces, and varies with species and weather conditions.

For an adequate understanding of the significance of transpiration reduction in interception of rainfall, a thorough analysis of heat transfer and water dissipation over natural wet and dry canopies is necessary.

## CONCLUSION

Evaporation and transpiration are energy dependent processes. A vegetative canopy wet with intercepted water has more energy available for evaporation by increased absorption of short-wave radiation, decreased emission in long-wave radiation, and a decreased or even negative sensible heat flux between canopy and the adjacent air. With more available energy, and a much lower diffusive resistance at the evaporation surface, evaporation of intercepted water occurs at a greater rate than transpiration under similar atmospheric conditions. So long as intercepted water is available for evaporation on a leaf's surface, transpiration is reduced or inhibited. Thus intercepted water is not a complete loss to the hydrologic cycle. Measurement of these two types of water loss has proved difficult, and the relative importance of reduced transpiration, especially under field conditions, is uncertain. Obviously, however, it is too large to be ignored in precise studies.

The variability of results obtained by different investigators illustrates the difficulty of measuring the relative rates of water movement in vapor state, and the multiplicity of factors affecting them. These problems also suggest the need for highly instrumented research designed to measure such rates under natural conditions and within specific vegetative types, together with data on temperature, humidity, radiation patterns, energy flows, wind movement, and rainfall characteristics. Intensive study of the interaction of transpiration, interception, and evaporation with these and other factors will be essential to an accurate understanding and evaluation of interception losses in forests and similar vegetative types.

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# CRITICAL LEVELS OF PHOSPHORUS AND NITROGEN IN TEXAS IMPOUNDMENTS[1]

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## INTRODUCTION

The 1972 amendments to the Federal Water Pollution Control Act require that each state classify its lakes and impoundments with respcet to the degree of aquatic plant fertility. Further, this Act requires the state to develop a plan for remedial action for those bodies of water that are determined to be excessively fertile. Texas, as well as other states, is in the process of examining the available information on nitrogen and phosphorus loadings, the sources of these nutrients, and the resultant concentrations in the State's impoundments. Further, they are attempting to assess the effect of these loads on water quality in the impoundments and the element(s) or other factors that exert primary control on aquatic plant growth in the water body. In the State of Texas the responsibility for making this assessment falls with the Water Quality Board. This organization must develop and implement an aquatic plant nutrient management plan which will restore water quality in those impoundments that are determined to be receiving excessive loads of the critical nutrient(s) that control aquatic plant growth. The results of these evaluations could be of great significance to the State of Texas in that aquatic plant nutrient control could cost State residents very large amounts of money for advanced waste treatment and altered agricultural and urban practices. Because of the large amounts of funds needed for aquatic plant nutrient control, it is important that the evaluation of the process governing algal growth be carefully done in order to develop technically sound, economically feasible managament plans for the problems of excessive fertilization of Texas' impounded waters.

One of the most critical aspects of the evaluation process is the determination of the critical levels and the loads that cause these levels of aquatic plant nutrients in Texas' impoundments. Because of the significance of this topic to the residents of Texas, it is important to discuss the approach that should be used to establish critical loading criteria for Texas' impounded waters. Presented below is a discussion of the information available on critical levels of nitrogen and phosphorus in the State's impoundments.

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*Previous Studies on Critical Levels of Aquatic Plant Nutrients*

The classical work on the critical concentrations of aquatic plant nutrients was conducted by C. N. Sawyer in the 1940's. Sawyer studied a group of Southern Wisconsin lakes and concluded that whenever the phosphorus exceeded 0.01 mg/1 P and the inorganic nitrogen exceeded 0.3 mg/1 N the lake was likely to have deteriorated water quality in the summer due to excessive algal and other aquatic plant growth.

Dr. R. A. Vollenweider has reviewed the data obtained on several European lakes and concluded that the 0.01 mg/1 P and 0.3 mg/1 N is appropriate for these lakes. The author has spent 13 years in Wisconsin studying problems of excessive fertilization of Wisconsin and other mid-western lakes and impoundments. As a result of these studies, he generally agrees with Sawyer on the critical levels of nitrogen and phosphorus in Wisconsin waters. During the past year, however, the author has had the opportunity to review the information on nutrient loads-algae response in Texas impoundments and concludes that at the present time there is insufficient information to establish critical nitrogen and phosphorus loadings or in-lake concentrations for these waters. While the critical concentrations cannot be established, it is clear that it is not technically sound to apply the 0.01 mg/1 P and the 0.3 mg/1 N criteria to Texas impoundments without a thorough evaluation of these criteria. The basis for this judgment is presented below.

*Water Quality and Water Use*

The Sawyer criteria are based on subjective judgment of impaired water quality due to excessive algal and other aquatic plant growth in the lakes that he studied. The large number of small lakes in Wisconsin affords the opportunity for someone to readily compare water quality in lakes within a relatively short distance. Within one day's drive, an individual in Wisconsin can observe lakes with almost distilled water quality which are essentially free of aquatic plant growth to extremely fertile lakes where ducks and turtles can walk on the algal scum that accumulates along the shore. Texas impoundments also have a wide variety of water quality although rarely are waters encountered that would be classified as oligotrophic by Wisconsin criteria.

From an aesthetic point of view, deep, crystal-clear water where it is possible to see the bottom in 30 to 40 ft. of water is highly desirable; however, a lake with this type water is generally so nutrient-poor that fish growth is seriously impaired and, as a result, a poor fishery is present. It is readily apparent in both Wisconsin and especially in Texas that a lack of high degree of water clarity is not a significant detriment to extensive recreational use of waters.

An important difference between Wisconsin lakes examined by Sawyer and many Texas impoundments is the turbidity of the water. In Wisconsin high water turbidity (reduced water clarity) is generally due to algal growth. In Texas, however, some impoundments have high level of turbidity due to erosional material. This background turbidity permits a higher level of algal growth

without serious impairment of water quality which would alter water-use patterns by the residents of the area. As discussed in the subsequent section, the erosional turbidity also affects nutrient load-lake response relationships in several additional ways.

It is clear from the information available that one of the first steps that must be taken to develop meaningful aquatic plant nutrient criteria for Texas surface waters is a study of the relationship between algal and other aquatic plant problems in Texas impoundments and the use of this water for municipal and industrial water supplies and recreational activities. This study should focus on the question of what is the aquatic plant nutrient load that causes a significant water quality deterioration to the water users of the area. This type of information is not available today, and it is certainly inappropriate to apply Wisconsin criteria to Texas impoundments.

*Cycling and Transport of Nutrients*

The Sawyer aquatic plant nutrient criteria are applicable to Wisconsin lakes nutrient concentrations at 'ice out' in the spring. It is inappropriate to apply these criteria to nutrient concentrations in Wisconsin lakes at other times of the year without a careful review of the characteristics. At the 'ice out' period in Wisconsin lakes and in other North American lakes that experience extensive ice cover, the nutrient concentrations are at a maximum in their available form. On the other hand, warm-water lakes such as Texas impoundments do not experience the ice cover and, therefore, do not have the long winter period of slow mineralization under the reduced light conditions which bring about the accumulation of nutrients in the spring of the year. In general, this could mean that the overall cycling of nitrogen and phosphorus in Texas impoundments would tend to be faster, and therefore, with those waters where the morphology, thermal regime, and hydrology are favorable, the lake would tend to utilize these nutrients to a greater extent and produce more algae per unit mass of nutrient input. However, to counter this is the fact that each time a unit mass of nutrient passes through the aquatic plant system, a certain fraction becomes refractory and, therefore, unavailable for future growths of algae.

In September 1973, an international workshop on warm-water lakes was held in Israel where it was concluded by the participants that warm-water lakes, such as those in Israel, Africa, and the southern U.S., utilize their aquatic plant nutrients differently than the cold temperate lakes, which have generally been studied in previous limnological investigations. There was general agreement that there was an urgent need for studies on nutrient flux-algal response relationships in warm-water lakes. The author fully supports this conclusion, and, based on his review of the information available on Texas impoundments, an extensive series of studies on nutrient transport and cycling must be made to determine how much algae and other aquatic plants develop per unit mass of aquatic nutrient input. If Texas is to properly manage these waters, considerable

amounts of federal and state funds will have to be appropriated to conduct the studies necessary to develop technically sound management policies.

*Turbidity, Water Quality and Algal Growth*

In addition to affecting how the public perceives algal growth in Texas impoundments, the turbidity due to erosional materials in these waters could have a pronounced effect on how a certain nutrient load affects algal growth in the impoundment. Turbidity affects light penetration with the result that less algal growth will occur per unit mass of nutrients in turbid lakes and impoundments than in clear lakes. Also, the turbidity typically present in Texas impoundments would tend to make nutrients less available by sorption of certain forms of the nutrients onto the surface of the solid particles. In addition, a substantial part of the nutrient load that enters Texas impoundments is in a particulate form, an unknown part of which is available for algal growth. There is need for a study on Texas impoundments in order to determine what fraction of the total nitrogen and phosphorus entering the water body can become available for algal growth and how much of this available nutrient is actually utilized within the water body.

## CONCLUSION

Based on the experience of the author, after review of the information available on aquatic plant nutrient water quality problem response in lakes and impoundments throughout the U. S., it is concluded that there is insufficient information available today to establish critical nutrient concentrations or loads for Texas impoundments. Because of the importance of these criteria in developing water quality management plans for these waters, it is recommended that no attempt be made to develop criteria at this time without additional study. These criteria must be based on the situations that develop in the Texas impoundments. Consideration must be given to the public's response to water quality in this region, the recreational and other uses of the waters as a function of algal and other aquatic plant growths, and the limnological and environmental chemical aspects of nutrient transport and cycling within these waters.

# WOLVES, COYOTES, DUCKS, AND HYBRIDISM

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## INTRODUCTION

We of the Texas Academy of Science are indeed fortunate to have the *Dialectic* section in our journal where we may share very speculative thoughts. In keeping with this "food for thought" vein, I wish to present a possible triggering mechanism for hybrid swarming. One of the most fascinating examples of range adjustment and/or usurpation, in my opinion, is that of the American Red Wolf, *Canis niger.* During the early part of this century, the coyote (*Canis latrans*) and the Red Wolf had a minimum of range overlap. The coyote prefers the more-or-less open prairies and the Red Wolf the more humid woodlands of the southeast.

In 1962, McCarley called attention to the replacement of true Red Wolves in much of East Texas by forms intermediate between and presumably hybrids between them and the coyotes. Paradiso and Nowak (1971) developed a technique to distinguish Red Wolves from coyotes on the basis of skulls and then proceeded to evaluate the taxonomic status and adjusted ranges of Red Wolves. This was based on the extensive collections at the U.S. National Museum, Washington, D.C.

The changing zoogeography of this endangered species was plotted (see Figure 1) as follows: In the northern and southern (coastal) areas of range abuttment between the 2 species, little or no hybridization occurred prior to 1930, and a pocket of nothing but hybrids separated the pure coyotes' range from that of the Red Wolf in southcentral Texas. Sometime after 1932 and at least by 1942 the population of hybrids had expanded northward and eastward until it had replaced pure Red Wolf populations in Oklahoma, Arkansas, and eastcentral Texas, leaving the only areas containing pure Red Wolves in southeast Texas, Louisiana, and possibly eastern Missouri. Range retraction of Red Wolves continued accompanied by replacement by hybrids until 1963-1969 when pure Red Wolves could be found only along the Gulf Coast in extreme southeast Texas and Louisiana. Before the work of Paradiso and Nowak (1971), it was recognized that hybrids had been replacing Red Wolves in East Texas but it was generally considered interbreeding of coyotes, that were expanding their range eastward, with the resident Red Wolves. It is now apparent, however, that interbreeding was not taking place in the northern and extreme southern portions of the species' range abuttment. Instead a viable population of hybrids (hybrid swarm) that probably had its origin

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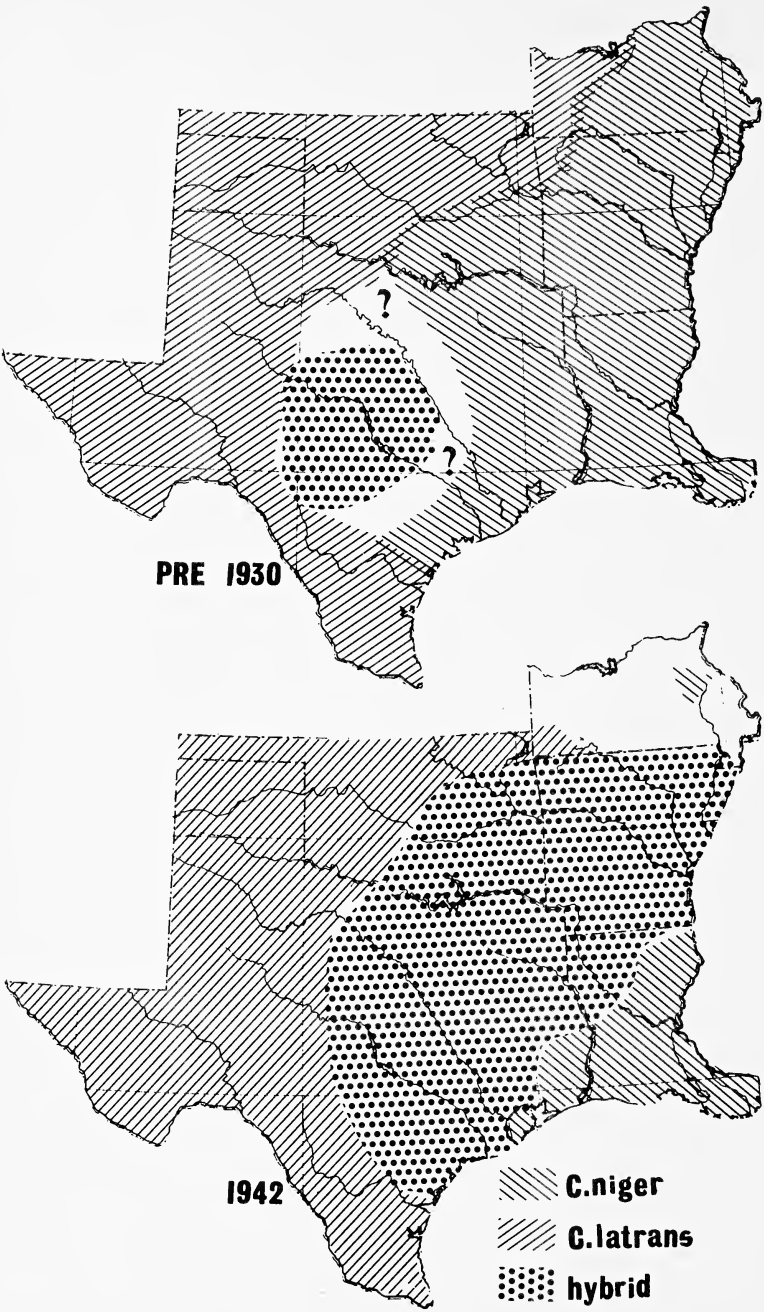


Figure 1. For precise numerical data and records by date and counties, see Paradiso and Nowak (1971), from which these maps were extrapolated.

in southcentral Texas was either usurping the Red Wolves' range or was rapidly replacing the Red Wolf as its numbers decreased. This probably resulted from man-induced changes in the range. The hypothesis seems well founded but as such events are very rare, the intriguing question concerns the triggering mechanism of such massive interbreeding in certain areas. The creation of these hybrid swarms is rare, but not because hybrids are inferior and unable to compete with parent types. For reasons of brevity, I shall not go into such things as hybrid vigor but it is well known that hybrids are often very competitive. Such happenings are rare because mating behavior is broken down and the hybrids do not continue to have genetic input to either of the parent populations. If, on the other hand, for one reason or another, an area produced an abnormal number of hybrids at a given time, those very competitive individuals could establish a very adaptive and therefore competitive population. But what would cause the sudden increase in hybridization?

Several years ago I began to observe ducks on a small (3 acres) artificial lake in Dallas, Texas, that is completely surrounded by homes. The fauna of the lake included a flock of 9 white Peking ducks, added 1 or 2 at a time after they grew out of the cute Easter duckling stage and a flock of 8 wild mallard ducks that had stopped by during fall migration and, no doubt enjoying the safety, stayed on to become permanent residents. The mallard ducks and the Pekings did not integrate, either socially or sexually. One Peking, that turned out to be a hen, had been ostrasized by the flock, apparently because of its runtiness and/or lack of feathers on the back of its head and neck.

With the introduction of the plastic loop 6-pac holders, that annually kill thousand of water fowl (mostly ducks and gulls), the ducks of this lake began to have the same problems of all other lake fowl. The dastardly "loops" that find their way into lakes and streams float and hold a deadly fascination to surface swimming birds. They peck at them until 1 or more loops are around their necks. Attempt at removal often winds additional loops either around the necks or feet until the duck either strangles, or is rendered easy prey, or starves. I captured all of these unfortunates that I could and removed the plastics. However, 1 handsome drake elluded me for over a week until he was starved down and could no longer offer resistance. During this time, his antics had caused him to be ostrasized by the remaining mallards and he had taken to swimming with the ostrasized Peking hen. After removal of the plastic, he remained with the white hen.

The following spring, one clutch each was produced by the Peking flock and the mallard flock as usual. Also, as usual, all ducklings were gone within a week due to predation by local domestic cats. The ostrasized couple also produced a clutch, 2 drakes of which survived. For some unknown reason, the following fall, 1 of the 2 mallard hens took up with the ostrasized Peking hen and mallard and the two hybrid male offspring of the previous season. So, within 2 years the "ragtag" flock surpassed both the mallard and Peking flocks which had remained stable due to unsuccessful survival of offspring. I hesitate to speculate on the reason for the apparent more successful survival of cat preditation although I am tempted to think along the lines of "hybrid vigor" and other rather vague adaptive phenomena.

Could it be that some sudden event causing an abnormal injury rate, not affecting viability, caused enough individuals of coyotes and Red Wolves to be ostrasized and cling together and start a hybrid population, or "swarm"? Increased use of steel traps by government trappers might be one candidate.

There are other possible reasons for such an event. Habitat changes in the area due to farming techniques, dam building, etc., but such changes were taking place all along the coyote-Red Wolf range abuttment and mixing was minimal. Apparently swarming did not take place during this period, while the area was inhabited by Indian populations, or before man moved into this area. The triggering mechanism (unidentified as of now) for the production of the hybrid swarm appeared sometime during the 3rd decade of the 20th century. In any case, these observations are offered as "food for thought" and with an invitation to comment or share the reader's own observations and ideas on this fascinating subject.

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PROCOELUS VERSUS OPISTHOCOELUS VERTEBRAE — Evolutionary selection for procoelus or opisthocoelus vertebrae (ball-and-socket) over amphicoelus (concave-concave) or platycoelus (flat ended) vertebrae is obviously to increase flexibility. Selection for procoelus over poisthocoelus, on the other hand, is not so easily explained. The vertebrae of modern crocodillians are procoelus (i.e..ball-and-socket with ball to the rear). They are aquatic and use their tails for propulsion through the water. Troxell (1925) analyzed the mechanics of crocodile vertebrae and concludes the procoelus has 2 distinct advantages over opisthocoelus vertebrae for this function. One, although not the first presented by Troxell, is that the column of procoelus vertebrae allows greater action for the ammount of angular movement. He states that bending a procoelus vertebral column describes an arc of smaller diameter than bending a column of opisthocoelus vertebrae. Our analysis disagrees with Troxell's and suggests that they produce arcs of equal radii.

The other advantage offered by Troxell, protection of the cup, appears valid. When a crocodile is swimming forward, the tail is the means of propulsion *and* the means of force transmission. Analysis of the procoelus caudel vertebrae demonstrates that the propulsive force is transmitted along its axis to the caudel next most anterior, which in turn duplicates the action by transmitting the received force along its axis to the caudel in front of it. Figure 1 shows forward force transmission in procoelus vertebrae versus that of opisthocoelus vertebrae. Force transmission from the cup onto the ball appears mechanically more sound than transmission from the ball into the cup. The danger of shearing the rim of the cup is increased if the cup is the receiver. Our investigation of this possibility involved several alligator vertebrae mounted on epoxy bases at various angles. They were tested for fracture tendencies under loads from a hydrolic press. The results demonstrate that a configuration where force is directed axially from the cup onto the ball can withstand 3 times greater pressure than one where force is transmitted from the ball into the cup. When the ball was advanced axially into the cup, the cup's rim sheared off. On the other hand, when the cup was advanced onto the inclined ball 50% more strokes of the hydrolic pump were required to break the vertebra. Furthermore, when it did break, it broke in the center. (compressibility of the green bone made accurate recording of the absolute force required impossible)

Animals with relatively long necks (sauropod dinosaurs, camels,giraffes, etc.) which hold their heads erect or nearly so, commonly have opisthocoelus cervical vertebrae. Even bird's cervical vertebrae might be considered opisthocoelus although the "ball" is more of a cylinder. These animals offer an interesting analogy with the mechanical analysis of crocodile vertebrae. In this case, the force acting along the cervical vertebrae is the acceleration of gravity which is directed down the column (to the rear) toward the body. As in the crocodillian tail the force is transmitted axially through the cup onto an inclined ball.

Our conclusion is that selection for opisthocoelus, or procoelus vertebrae is dependent upon the direction of forces applied to the vertebral column and that protection of the cup rim is the primary concern.

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*–William D. Gosnold and Bob H. Slaughter, Shuler Museum of Paleontology, Southern Methodist University, Dallas 75221.*

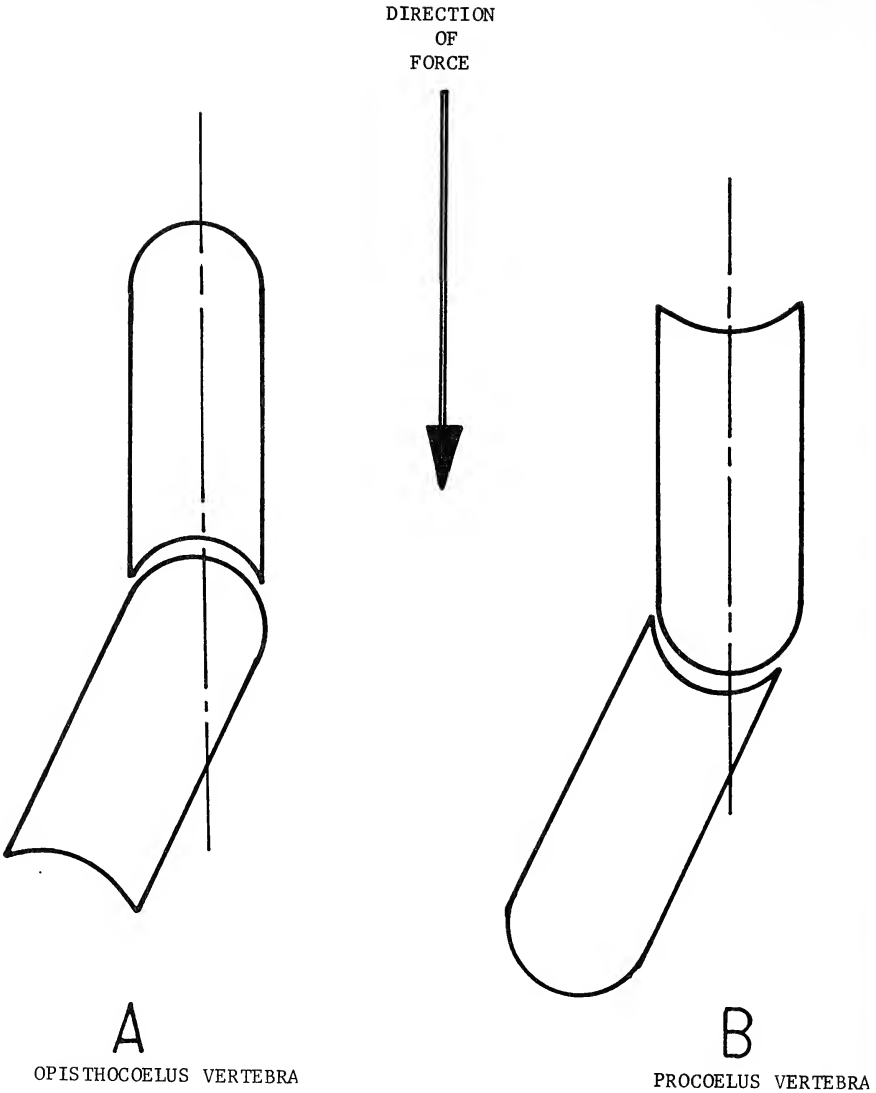



Figure 1. Schematic drawing showing opisthocoelus vertebrae (A) and procoelus vertebrae with force transmission from the same direction.

A NOTEWORTHY RECORD OF THE SILVER-HAIRED BAT IN SOUTHEAST TEXAS — On the evening of 24 September 1975, a silver-haired bat, *Lasionycteris noctivagans,* was captured in a residential area near the intersection of 69th Street and Stewart Road in the city of Galveston, Galveston County, Texas. The specimen (Texas Cooperative Wildlife Collection No. 29002) was an adult female weighing 13.6 g. External measurements in mm were: total length, 101; tail, 40; hindfoot, 10; ear from notch, 16; forearm, 42.

This is the southeasternmost record of *L. noctivagans* in Texas and the first occurrence of the species in the Gulf Coast prairie and marsh region of the state. McCarley (1959, *Texas J. Sci.*, 15:385) did not report the silver-haired bat as part of the East Texas bat fauna. The nearest records to the Galveston locality are from Medina County, 250 mi to the west on the Edwards Plateau (Blair, 1952, *Texas J. Sci.*, 4:95), and from Winn Parish, approximately 300 mi to the northeast in the pine-hardwood forest of north-central Louisiana (Lowery, 1974, The Mammals of Louisiana and its Adjacent Waters, Louisiana State Univ. Press). Other documented localities from Texas include those from Lubbock, Hockley, and Culberson counties (Davis, 1975, *The Mammals of Texas, Bull.* 41-rev., Texas Parks and Wildlife Dept.).

According to Davis (*op. cit.*), *L. noctivagans* is migratory, spending the summer in northern latitudes and wintering toward the south, but wintering locations are largely unknown. All specimens taken from Texas, except for 17 June males captured in the Guadalupe Mountains by LaVal (1973, *Southwestern Nat.*, 17:357), probably represent migrating individuals (Davis, *op. cit.*). The Galveston specimen was probably also migrating; this was suggested by its September occurrence in an atypical environment south of its normal range and by heavy layers of fat found on the specimen. The discovery of *l. noctivagans* on the Texas coast during September and a recent January record of the species in the San Carlos Mountains of Tamaulipas, Mexico (Schmidly, *In Press, J. Mamm.*) may indicate that certain populations migrate through Texas to winter in the forested regions of Mexico.

I would like to thank David J. Schmidly for reviewing the manuscript and making helpful suggestions. Special thanks goes to Bill Proctor of Galveston, Texas, who captured the specimen and donated it for my examination.—*Chester O. Martin, Environmental Resources Branch, U.S. Army Corps of Engineers, Galveston District, P.O. Box 1229, Galveston 77550.*

OCCURRENCE OF STRIPED BASS (*MORONE SAXATILIS*) IN COASTAL WATERS OF TEXAS—A commercial net fisherman caught a striped bass in the Gulf of Mexico surf 1-1/4 mi east of the North Galveston Jetty, Texas, on 21 January 1975. He sold the fish to James Hornbeck, the proprietor of Milt's Seafood (Port Bolivar, Texas), who notified officials of the Texas Parks and Wildlife Department. The authors examined the specimen.

The dressed weight was 5.35 kilograms (14 pounds). The total length was 80.5 cm (31.7 in); standard length was 68.0 cm (26.8 in). Spine and ray counts were; first dorsal dorsal IX; second dorsal I, 12; anal III, 11; pectoral 15; pelvic I, 5. The testes are preserved at the Parks and Wildlife Department Laboratory, Seabrook, Texas. We examined scales from its left side near the tip of the pectoral and above the lateral line. There appeared to be 6 to 7 "annuli" on each scale.

To our knowledge this is the second authentic occurrence of a striped bass in the coastal waters of Texas. E. G. Simmons (Parks and Wildlife Department, Rockport, Texas, personal communication) examined a 3 lb specimen caught off the south jetty at Port Aransas, Texas during the late 1960s. Talbot (1966, *Amer. Fish. Soc., Spec. Publ.* No. 3:37) reports the occurrence of striped bass in major streams of the Gulf of Mexico from the Florida coast to the Tchefuncta River, Louisiana. Baughman (1950, *Texas J. Sci.*, 2:242) reported that its presence in Texas is doubtful; however, he cited Taylor (1878, *Forest and Stream*, 2:7) who reported *Roccus saxatilis* in Texas. It is not listed by Hoese (1958, Publs. Inst. Mar. Sci., 5:312) and Parker (1965, *Publs. Inst. Mar. Sci.*, 10:201).

Parks and Wildlife Department biologists have stocked fry in fresh waters of Texas since 1967 and the Louisiana Wildlife and Fisheries Commission stocked fry in Lake D' Arbonne and Toledo Bend (Hughes and Walker, 1974, *La. Conservationist,* 26:18).–*R.L. Benefield, A.W. Moffett, and L.W. McEachron, Parks and Wildlife Department, Seabrook 77586.*

A SURVEY OF ECTOPARASITES OF HARES AND RABBITS IN GRANT COUNTY, NEW MEXICO– In 1934, Ward (1934, *Proc. Okla. Acad. Sci.,* 14:31) published a study of rabbit parasites in central Oklahoma. Randolph and Eads (1946, *Ann. Entomol. Soc. Amer.,* 39:597) and Stannard and Pietsch (1958, *Nat Hist. Sur. Div. Biol., Notes,* 38) listed some ectoparasites from *Sylvilagus Floridanus* in Texas and Lee County, Illinois, respectively. In New Mexico, Morlan (1955, *Texas Rep. Biol. Med.,* 13:93) mentioned fleas from *Lepus* and *Sylvilagus* taken during a survey of mammals of Santa Fe County. Likewise, Kartman (1960, *Zoonoses Res.,* 1:1) included information on the ectoparasites of both these lagomorpha in eastern New Mexico.

The purpose of this study was to examine ectoparasites of *Lepus* and *Sylvilagus* in a different region and environment in southwestern New Mexico. Two species of lagomorph, *Lepus californicus* Gray, the blacktailed jack rabbit, and *Sylvilagus auduboni* (Baird), the desert cottontail, were collected from the Upper Sonoran life zone of Grant County, New Mexico. The jack rabbit is associated with grasslands and is also found in areas of pinon-juniper and of oak woodland. The cottontail is more abundant in woodlands of the foothills and elevated mesas in the central area of Grant County. Twenty-eight cottontails and 14 jack rabbits were collected and examined for ectoparasites.over a span of 8 months at localities selected on the basis of relative host population densities and distributions. The parasites were preserved in 95% alcohol, treated with 10% potassium hydroxide, 0.02 N HCL, and beechwood creosote then mounted in Canada balsam.

Ninety-eight percent of the lagomorpha were infested with ectoparasites. Five species of fleas, 3 species of ticks, and a single bot-fly larval species were recovered. The fleas *Cediopsylla inaequalis inaequalis* (Baker) and *Hoplopsyllus glacialis affinis* (Baker) were recovered from 2 and 26 desert cottontails, respectively. *Echidnophaga gallinacea* (Westwood) which is usually found on birds, and *Meringis dipodomys* Kehls, common on wild rodents, proved to be incidental fleas of this species of rabbit. Thirteen *H. glacialis affinis* and 5 *E. gallinacea* were taken from 6 jack rabbits. Adult *Haemaphysalis leporis-palustris* (Parckard), was found on only 2, or 7%, of the jack rabbits but was more abundant on cottontails (21%). However, 13, or 93%, of the jack rabbits examined were infested with the ticks *Dermacentor parumapertus* Neumann. Two adults and 1 nymph of *Otobius megnini* (Duges) were collected from 2 different cottontail hosts but were not found on jack rabbits. Only 5 larvae of *Cuterebra* were taken from 3 different cottontail host. Based on distribution records, these bot-fly larvae are probably *C. cuniculi* (Clark) or *C. leporivora* Coquillett. These observations compare favorably with results obatined by Ward (1934) in Oklahoma, Randolph and Eads (1946) in Texas, Morlan (1955) in Santa Fe County, New Mexico, and Stannard and Pietsch (1958) in Lee County, Illinois.

The author wishes to thank Professor C. Clayton Hoff for his advice and suggestions during this study. – *Paul H. Rodriguez, Division of Allied Health and Life Sciences, University of Texas at San Antonio, San Antonio 78285*

A KIAM INCISED POT FROM LOUISIANA – The Central Louisiana Electric Company cleared for a new power line right-of-way last year on the Louisiana side of

Toledo Bend lake and bulldozed out a crushed Indian pot. Mrs. Melva L. Marsh recovered the pieces. The John Guy fmaily at Anacoco put me in contact with Mrs. Marsh and I undertook reconstruction of the pot with fair results and a lot of experience. About 2/3 of the vessel was present.

The pot was found on a hilltop in Sabine Parish. Coordinates are 45.1 NS-346.2 EW, U. S. Geological Survey, Alexandria Series, V502; Add. IAMS, NH 15-2. There was no indication of a burial present but occupational debris is scattered over the surface. It is unusual to find this large a vessel in this much completeness.

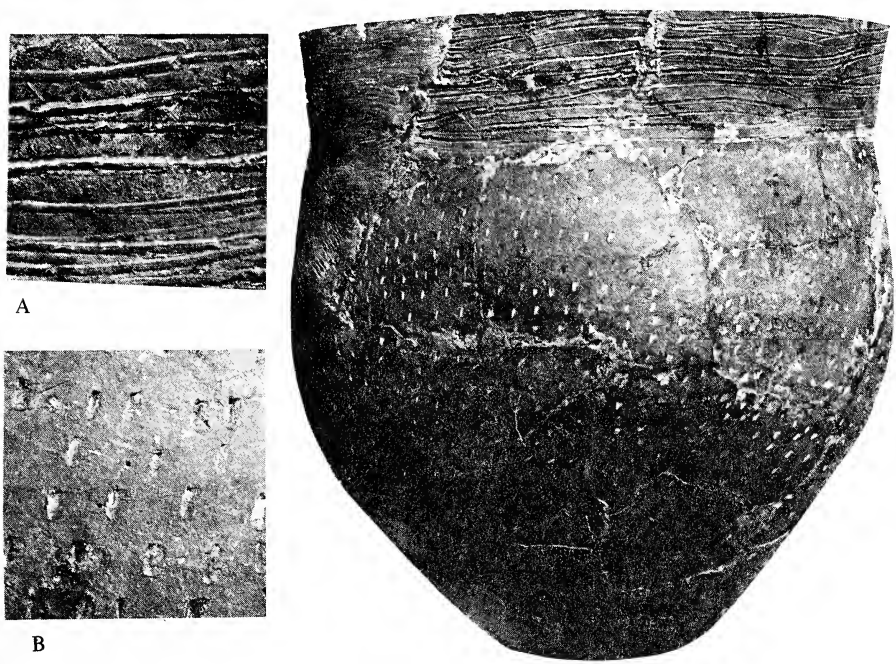
The restored is 37.46 cms high (14.75 in). The mouth is 33 cms (13 in) on the outside. The greatest width is 38.1 cms (15 in), 17.78 cms (7 in) above the base. The bottom is slightly concave and is 12.7 cms (5 in) wide. The neck is slightly flared. The capacity is 25.12 l (6.64 gal.). The color is reddish brown with darker areas near the base.

The temper is clay and grit with some fiber. Thickness of the wall averages 7.94 mm (5/16 in). The rim is rounded and flush with the sides.

The decoration consists of irregularly incised lines around the neck. Punctations, made with a small wedge-shpaed object, begin 12.7 cms (5 in) above the base and cover the whole pot up to the neck. The finish is fine on the lower part and coarser above.

According to Suhm and Jelks (1962, *Editors Handbook of Texas Archaeology: Type Descriptions.*, The Texas Archaeological Society and The Texas Memorial Museum, Austin, Texas), this vessel is a Kiam Incised pot belonging to the Alto Focus and dated between 500 and 1000 A. D.. McClurkan, Field, and Woodall (1966, *Excavations in Toledo Bend Reservoir,* 1964-65., Papers of the Texas Archaeological Salvage Project, No. 8, Austin, Texas.), found sherds of Kiam Incised at the Salt Lick Site, 7 mi west of Many, also in Sabine Parish.

The vessel is in the personal collection of Mrs. Melva L. Marsh, RR3, Box 62, Florien, Louisiana, 71429. Photographs by Steven Lewis.–*Russell J. Long, Department of Biology, Lamar University, Beaumont 77710.*



A

B

Figure 1. Kiam incised pot. A, detail of neck; B, detail of body.

RESOLUTION OF 2-(*O*-CHLOROPHENYL)-2-(*P*-CHLOROPHENYL) ACETIC ACID—Mitotane is best known by its trivial name, *o,p'*DDD, and is chemically 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane. This drug is used in the treatment of inoperable adrenal cortical carcinoma of both functional and non-functional types (Hutter, 1966, *Amer. J. Med.*, 41:575).

Mitotane can best be described as an adrenal cytotoxic agent, although it can cause adrenal inhibition, without cellular destruction. Its biochemical mechanism of action is unknown. Mitotane has been shown to inhibit adrenal glucose-6-phosphate dehydrogenase in the dog and corticotropin stimulation of steroidogenesis *in vivo* but not *in vitro* (Hart, 1971, *Biochem. Pharmacol.*, 20:1679; Grady, 1965, *Proc. Soc. Exp. Biol. Med.*, 119:238). These findings suggest that a metabolite of miotane rather than the compound itself is responsible for its activity. The major metabolite of mitotane appears to be 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)acetic acid, *o,p'*DDA (Sinsheimer and Guilford, 1972, *J. Pharm. Sci.*, 61:314). We wish to describe an improved preparative method for the synthesis of 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)acetic acid, *o,p'*DDA, and its chemical resolution.

A mixture of 80 ml diethylene glycol and 10.0 g of 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane was added to a solution of 12.6 g of KOH in 7 ml of water. The mixture was stirred for 4 days at 110 C. The mixture was allowed to cool and was poured, with vigorous stirring, into 250 ml of cold water. The insoluble material was filtered and washed twice with 10 ml portions of warm water. The filtrate was boiled gently for 5 min with 2 g of decolorizing carbon; the carbon was removed by filtration. The filtrate was acidified to litmus with 20% $H_2SO_4$ (*ca* 50 ml), and then an additional 6 ml of the acid was added. The mixture was cooled in an ice bath for 15 min. The precipitate was collected by suction filtration, washed free of sulfate ions with water, dried at 85 C, recrystallized from methanol, and gave 3.5 g of product (44% yield, mp 104-105 C). The old method involved a $Ba(OH)_2$ hydrolysis (Cristol and Haller, 1945, *J. Am. Chem. Soc.*, 67:2222) and gave a yield of 13% (lit. mp 107-108 C corrected).

*o,p'*DDA (2.0 g) was dissolved in 40 ml of ethanol with warming to 50 C. Quinine (2.3 g) was added over a period of 10 min. The solution was heated to boiling and 25 ml of water was added (to turbidity). The mixture was allowed to cool to room temperature and stand for 2 days. The product was recrystallized from methanol-water to constant rotation, air dried at 60 C to yield 1.3 g, mp 156-157 C; $(\alpha)\ ^{25}_{D}$ (ethanol)-22.5 (c 2.48).

A 2-ml portion of concentrated HCl was added to a mixture of 1.3 g of the diastereoisomer and 40 ml of water. The solid precipitate was filtered and washed with 5 ml portions of 50% HCl, recrystallized from ethanol to constant rotation, air dried and then dried at 80 C to give 0.470 g of *o,p'*DDA, mp 105-106 C, $(\alpha)\ ^{25}_{D}$ (chloroform) + 11.5 (*c* 1.00); $\upsilon$max (KBr) 3000-2500 (OH 1710 (C=O) $cm^{-1}$. The essential data for racemic *o,p'*DDA (III), appear in Table 1.

TABLE 1

Intermediates in the Resolution of 2-(*o*-Chlorophenyl)-2-(*p*-Chlorophenyl) Acetic Acid and (-)-2-(*o*-Chlorophenyl)-2-(*p*-Chlorophenyl) Acetic Acid.

| No. | M.P. C | Formula | C | H | N | C | H | N |
|---|---|---|---|---|---|---|---|---|
| I | 104 - 105[2] | $C_{14}H_{10}O_2Cl_2$ | 59.80 | 3.58 | – | 59.90 | 3.60 | – |
| II | 156 - 157 | $C_{34}H_{34}N_2O_4Cl_2$ | 67.43 | 5.66 | 4.62 | 67.52 | 5.56 | 4.51 |
| III | 105 - 106 | $C_{14}H_{10}O_2Cl_2$ | 59.80 | 3.58 | – | 59.89 | 3.52 | – |

1. Melting points are uncorrected.
2. A mixed melting point with an authentic sample showed no depression.

This work was supported by the National Institutes of Health (Grant RR-08061) through the National Cancer Institutes—*J. Guilford, E. Hickman, and D. Ghosh, Texas Southern University, School of Pharmacy, Houston 77004.*

OBSERVATIONS ON THE ECOLOGY OF *MICROPTERUS TRECULI* IN THE GUADALUPE RIVER—Hook and line collections and skindiving observations were made of *Micropterus treculi* in approximately 28 km of the Guadalupe River from State Highway 473 (Sultanfuss crossing) to U.S. Highway 281 bridge, Kendall County, Texas, March 12 through 15, 1975. A representative sample of the catch was catalogued in the Oklahoma State University zoological museum following confirmation of identification by R.J. Miller.

During the daytime hours of this period, surface water temperatures ranged from 14 to 17 C and the maximum air temperature was 26 C under partly-cloudy skies. Underwater visibility estimates ranged from approximately 1 m in mainstream areas to 6 m in a spring-fed tributary stream. In upstream areas *M. treculi* were taken on spinners in riffle areas and swift water. Farther downstream where riffles were widely separated by deep pools, Guadalupe bass were caught in deep eddies in riffle tail races. Largemouth bass, *Micropterus salmoides* were taken only in deep pools, while no spotted bass, *Micropterus punctulatus*, were caught. Hurst, Bass and Hubbs, 1975, *Natl. Symp. on the Biol. and Mgmt. of the Centrarchid Basses*, 47-53, reported that "trophy" sized Guadalupe bass would be about 250 mm SL. This is approximately 310 mm or greater. Two Guadalupe bass were taken having a TL of 330 mm. The authors are aware of no information available on the movements of *M. treculi*. However, Gerking, (1953, *Ecology*, 34:347) reported a home range of 200 to 400 linear ft (approximately 61-122 m) for the closely related spotted bass *M. punctulatus* in a small Indiana stream. The stretch of river from which these specimens were taken was approximately 12.9 km to 40 km upstream from Canyon Reservoir. It therefore appears likely that the large specimens taken in our study were lentic residents and not upstream migrants which might have exhibited accelerated growth rates in a newly formed reservoir.

With 5 of 7 and 2 of 13 male *M. treculi* caught, it was possible to cause apparently viable sex products to be emitted from the genital openings by application of gentle pressure to the abdomen. Only 1 of 4 female and none of 4 male largemouth bass taken in the same period were similarly determined to be in "ripe" spawning condition. Hurst, Bass and Hubbs (1975) suggested a May through June spawning period for *M. treculi* based upon size-class evaluations and the presence of large ova in a 70 mm SL female collected in May. Our results, however, indicate a spawning period for large-sized Guadalupe bass beginning much in advance of this time.

In skindiving observations of several mainstream areas and the lowest pool areas of 3 tributary streams, no large-mouth or spotted bass nests were found. However, 1 actively guarded *M. treculi* nest was observed. The individual guarding this nest was approximately 280 mm TL and moved a short distance away when approached. A second individual, thought to be a spawning female was seen nearby beneath an overhanging creek bank. The nest site was located approximately 75 m upstream of the mouth of a springfed tributary at a depth of 69 cm. It was situated 1 m from the shore on a 5 to 10 degree slope with the current speed at this point being approximately 0.3 m per second. The nest was an oval shaped, bowl-like depression 51 cm by 43 cm and 10 cm in depth. It was swept into the hard black soil of the creek bank and was free of silt. The interior of the nest was lined with 5 cm diameter limestone rubble which was partially covered by a few scattered sticks and leaves. The eggs within the nest were adhered to the sticks and leaves with only a few on the tops and sides of the rubble. All eggs were collected in a cloth bag and preserved in 10% formalin. Later enumeration showed the nest to contain 1,406 eggs. This was fewer than the 5,016

average number of eggs reported by Vogele (1975, *U.S. Fish and Wildl. Serv. Tech. Pap.* 84:1) for nests of spotted bass in Bull Shoals Reservoir, Arkansas. The average diameter of 200 eggs from this *M. treculi* nest was 2.058 mm with a standard deviation of .078 mm. This was larger than the 1.93 mm average diameter reported by Vogele (1975) for eggs collected from nests of *M. punctulatus*. Stomachs of 5 *M. treculi* were preserved and later examined. Three were empty, 1 contained an immature aquatic insect, and the remaining 1 contained 1 crayfish and 2 partially digested *Notropis sp.*—*R.L. Boyer, G.W. Luker, and R.J. Tafanelli, School of Biological Sciences, Oklahoma State University, Stillwater 74074.*

AN AMBICOLORATE WINDOWPANE (*SCOPHTHALMUS AQUOSUS*), WITH NOTES ON OTHER ANOMALOUS FLATFISH—An almost totally ambicolored windowpane (*Scophthalmus aquosus*; Texas Cooperative Wildlife Collection 0419.1; 130 mm TL, 101 mm SL, 22 g) was trawled off Waccamaw Sound, South Carolina 16 May 1973 (Figure 1). Pigmentation similar to that of the ocular side covers approximately 75% of the right- or blind-side surface, with a majority of the head region, proximal portions of the first 8 dorsal fin rays and a wedge-shaped area extending dorso-posteriad from above the preopercle to the vicinity of the 27th dorsal fin ray exhibiting typical blind-side coloration. Premaxillary and dentary bones, exhibiting faint coloration on anteriormost portions, are the only regions of the head bearing pigmentation. Comparison of eyed- and blind-side coloration indicates no symmetry of pigmentation patterns.

Our specimen represents the first recorded instance of abnormal coloration in *Scophthalmus aquosus*. Pigment abnormalities in other species of the genus *Scophthalmus* are common (Hussakof, 1914, *Bull. Am. Mus. Nat. Hist.* 33:95; Norman, 1934, *British Mus. Nat. Hist.*, 1:22), especially among European species and particularly turbot (*S. maximus*). The piebald condition of the lower surface is so common among turbot that it was considered by early French zoologists as characteristic of the species (Giard, 1892, *Compt. Rend et Mem. Soc. Biol.*, 9(IV):31). Norman (*op. cit.*) found that although ambicolorate examples have been recorded for a number of scophthalmids, color anomalies seem to be much more common in certain species than in others. Norman demonstrated that total ambicoloration is commonplace in turbot and generally rare in brill (*S. rhombus*), a sympatric species. Our specimen increases the number of bothids exhibiting abnormal pigmentation and strengthens Norman's conclusion that ambicoloration is rarer in some species than in others.

The remainder of this paper describes several examples of pigment and associated morphological abnormalities in southern flounder (*Paralichthys lethostigma*) taken from Texas waters. A partially albinistic southern flounder (TCWC 4032.1; 255 mm TL, 205 mm SL, 168 g) was collected 3 December 1968 from the cooling-water intake canal of Houston Lighting & Power Company's P. H. Robinson Generating Station on Galveston Bay, Texas. Right-side coloration is typical of the species, while the left-side of the caudal peduncle and caudal fin is largely albinistic. Lack of pigmentation extends from the caudal peuncle area 25 mm anterior to the caudal fin base and through dorsal rays 85 to 92 and anal rays 70 to 73. Small, scattered splotches of color exist on the distal portion of the caudal fin rays. A transitional zone of partial pigmentation exists between normal and albinistic areas. Although no visible morphological abnormalities are evident, a radiograph (Figure 2) does reveal osteological malformation in the hypural plate region. The proximal portion of the first autogenous epural is lodged tightly between an abnormally thickened last neural spine (penultimate vertebra) and a ventrally-bowed, second autogenous epural. The second autogenous epural and first 2 epural elements of the urostyle are broken into approximately equal halves, with the distal halves dislodged ventrally. The base of the last haemal spine appears to have been notched posteriorly by ventral flexure of the urostyle. Location and extent of both color and osteological abnormalities in our specimen closely resemble those described by Dawson (1967, *Trans. Am. Fish. Soc.*, 96:400) for a 64 mm SL southern flounder. However, Dawson's specimen exhibits more extensive damage to the

Figure 1. Right side of an almost totally ambicolored *Scophthalmus aquosus* (TCWC 0419.1). Left side is normally pigmented.

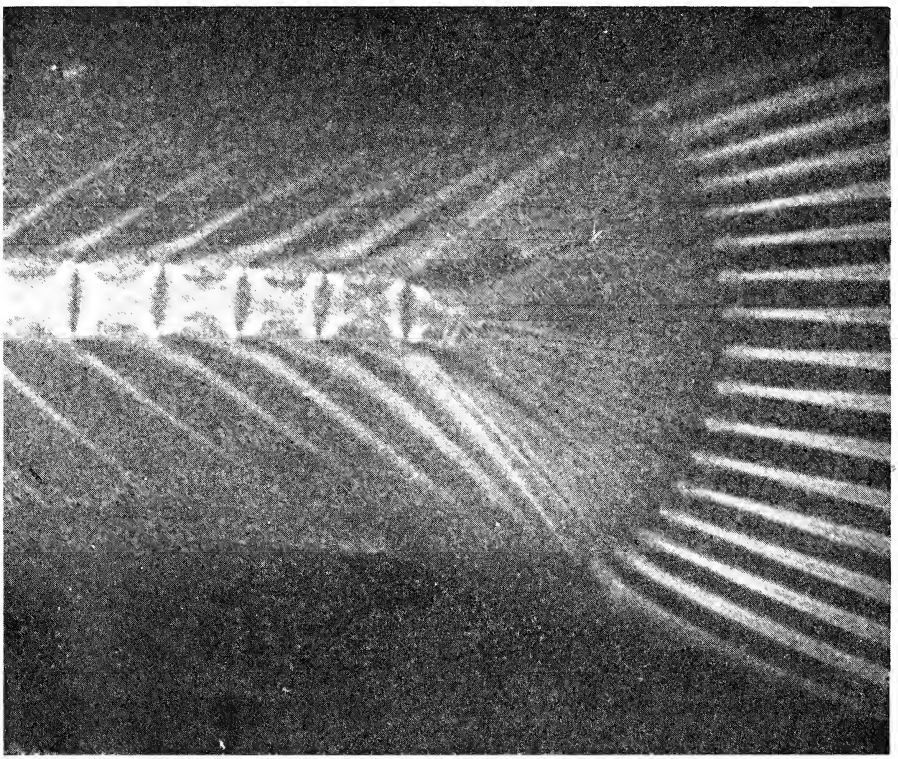


Figure 2. Radiograph of a partially albinistic *Paralichthys lethostigma* (TCWC 4032.1) illustrating osteological damage to the hypural plate region.

posterior caudal vertebrae and caudal fin and a proportionally larger albinistic area. Our findings and other records of albinistic flatfishes exhibiting morphological aberrations (summarized in Dawson, 1962, *Copeia,* 1962:138) provide supporting evidence for Norman's (*op. cit.*) theory that partial albinism in heterosomates is generally associated with osteological injury.

A totally ambicolored southern flounder (TCWC 0420.1; 318 mm TL and 266 mm SL) was trawled from East Matagorda Bay, Texas 23 October 1973. This specimen fits Dawson's (1962, *op. cit.*) Type D anomaly classification in exhibiting almost total ambicoloration, incomplete eye rotation and hooked dorsal fin. Typical pigmentation covers the entire blind side except for outermost portions of the pectoral fin and the lower, anteriormost point of the dorsal fin hook. The migrating eye is positioned atop the dorsal crest in an extensive depression of the frontal region and is in the same plane as the right edge of the dorsal fin and right nasal openings. Other atypical features exhibited by this specimen include variation in pelvic fin length and position toward symmetry and an anomalous concave posterior half of the left lateral-line arch. A radiograph of the head region reveals several osteological abnormalities (Figure 3). Median portions of the left frontal bone encompassing the right orbit exhibit an incomplete ventro-lateral migration and, as a result, are positioned along the dorsal midline. Premature arrestment of counter-clockwise twisting of this medial sector has caused the posterior oblique ridge of the right orbit to be

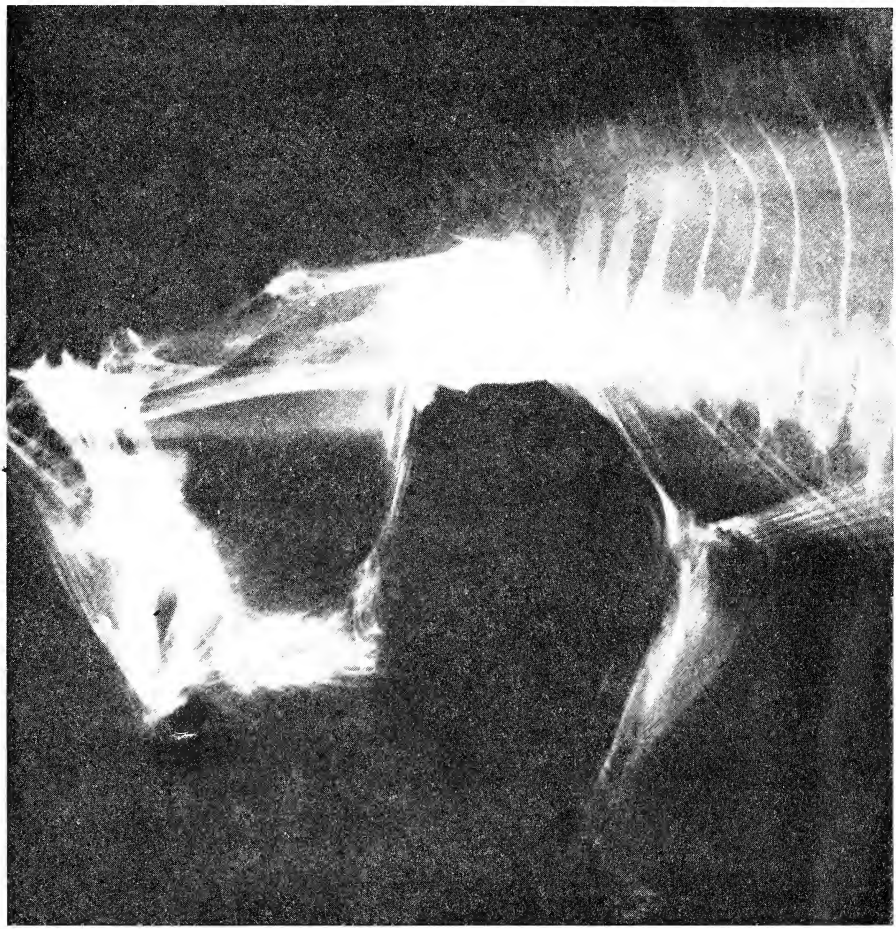


Figure 3. Radiograph of the head region of a totally ambicolored *Paralichthys lethostigma* (TCWC 0420.1) illustrating incomplete migration of left frontal bone.

directed dorso-posteriad instead of dorso-anteriad. This incomplete deflection of the central skull region also has inhibited forward growth of the dorsal fin and has resulted in crowding of anteriormost dorsal fin rays and associated pterygiophores between the posterior oblique ridge of the right orbit and the first neural spine. Although dorsal fin rays and pterygiophores are on a 1-to-1 basis, the first pterygiophore is in contact with the oblique ridge of the right orbit and does not function in direct support of the first dorsal fin ray. The radiograph of our ambicolorate flounder closely resembles that taken by Powell and Schwartz (1972, *J. Elisha Mitchell Sci. Soc.*, 83(3):155) of a totally ambicolorate southern flounder exhibiting hooked dorsal fin and rearward projection of the oblique crest of the left frontal bone. These radiographs support Norman's (*op. cit.*) finding that frontal bones arrive in final position only after eye migration is complete. Furthermore, it appears that,

although forward extent of dorsal fin growth in flatfish is dependent upon degree of eye migration, number of dorsal fin rays and pterygiophores is determined before complete eye rotation is attained.

An almost totally ambicolored southern flounder (TCWC 4033.1; 293 mm TL, 240 mm SL, 337 g) was taken 7 August 1972 in the Houston Ship Channel sector of Galveston Bay, Texas. This flounder exhibits pigmentation on approximately 85% of its blind side. The head, pectoral fin and portions of the first 13 dorsal rays exhibit normal blind-side albinism. Although blind-side pigmentation is slightly lighter than that on the eyed side, there is a sharp distinction between pigmented and non-pigmented areas on the lower surface. Except for a strongly convex blind-side contour, this specimen exhibits no other morphological or osteological abnormalities.

We wish to thank Houston Lighting & Power Company for financing the study during which the partially albinistic specimen was collected. We also wish to thank Messers. E.A. Bruguiere, W.L. Oliver, J. Poncik and H.A. Smith for making the anomalous specimens available to us.—*Andre M. Landry, Jr., Department of Marine Sciences, Texas A&M University, Galveston 77550, and Kenneth W. Johnson, Department of Biology, Mary Hardin-Baylor College, Belton 76513.*

ANALYSIS OF STUMP VEGETATION IN AN EAST TEXAS POND—Plants growing in and around water are becoming more significant as reservoirs are proposed and built and as more environmental impact studies are initiated. It is necessary, when discussing the effects a particular reservoir project might have on the vegetation present,

TABLE 1

Density, Frequency and Importance Value Data for Species Found Growing on Stumps in an East Texas Pond.

| Species | Density no/stump | Relative density % | Frequency % | Relative frequency % | Importance value* |
|---|---|---|---|---|---|
| *Juncus repens* | 7.5 | 33.9 | 47.5 | 12.6 | 46.5 |
| *Lycopus rubellus* | 4.0 | 18.1 | 45.2 | 12.0 | 30.1 |
| *Rhynchospora glomerata* | 2.3 | 10.4 | 36.8 | 9.7 | 20.1 |
| *Xyris difformis* | 2.2 | 9.8 | 35.2 | 9.3 | 19.1 |
| *Proserpinaca palustris* | .9 | 4.0 | 30.3 | 8.0 | 12.0 |
| *Ascyrum stans* | .8 | 3.4 | 20.1 | 5.5 | 8.9 |
| *Bidens polylepis* | .7 | 3.1 | 21.5 | 5.7 | 8.8 |
| *Aster lateriflorus* | .8 | 3.4 | 19.1 | 5.1 | 8.5 |
| *Myrica cerifera* | .4 | 2.0 | 23.0 | 6.1 | 8.1 |
| *Hypericum walteri* | .5 | 2.2 | 17.6 | 4.7 | 6.9 |
| Others** | | 9.7 | | 21.3 | 31.0 |
| Total | | 100.0 | | 100.0 | 200.0 |

* Sum of relative density and relative frequency.

** Other species recorded on stumps listed in order of decreasing importance value: *Andropogon virginicus, Panicum verrucosum, Pluchea foetida, Sium suave, Pinus taeda, Hydrolea ovata, Hydrocotyle verticillata, Cephalanthus occidentalis, Scirpus cyperinus, Cyperus haspan, Ludwigia glandulosa, Acer rubrum, Ludwigia palustris, Nymphaea odorata, Persicaria coccinea, Amorpha paniculata, Eupatorium perfoliatum, Liquidambar styraciflua, Paspalum longipilum, Rubus* sp., *Smilax* sp., *Bulbostylis ciliatifolia, Desmodium sessilifolium, Rhus copallina, Solidago rugosa, Baccharis halimifolia, Helianthus angustifolius, Lobelia cardinalis, Panicum agrostoides, Spiranthes laciniata, Mikania scandens.*

to submit possible immediate or long-term changes in the vegetation of that impoundment area. The object of this study was to provide information on plants likely to be found on logs and stumps in reservoirs or other aquatic habitats.

The pond studied was located near Nacogdoches, Texas. Three-fourths of the pond was surrounded by trees and shrubs whereas one side of the pond fronted a highway and is characterized by herbaceous species. Imperical observation indicated that loblolly pine (*Pinus taeda* L.), sweetgum (*Liquidambar styraciflua* L.), black gum (*Nyssa sylvatica* Marsh.) and red maple (*Acer rubrum* L.) were prevalent species bordering the pond while shortleaf pine (*Pinus echinata* Mill.) and white oak (*Quercus alba* L.) were occasionally present. Local populations of common buttonbush (*Cephalanthus occidentalis* L.) and wax myrtle (*Myrica cerifera* L.) were also observed.

There were 161 stumps in the pond. Plants present on each stump were identified and counted and density and frequency data were obtained. Counts for rhizomatous species included individual shoots or plants along each rhizome. Nomenclature followed Correll and Johnston (*1970; Texas Research Foundation, Renner, Texas*). Stumps were periodically sampled from March to October.

Forty-one species of plants were found growing on the stumps. These species are listed in order of importance value in Table 1. Penfound (1949, *Proc. La. Acad. Sci.* 12;47) recorded 26 species of plants on logs in Lake Chicot located in Louisiana. Ten of these species were found on stumps in our study.–*E.S. Nixon, W.F. Trotty, B.L. Bates, and D.L. Wilkinson, Department of Biology, Stephen F. Austin State University, Nacogdoches 75961.*

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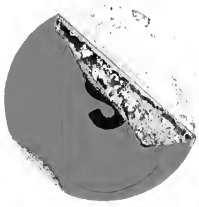

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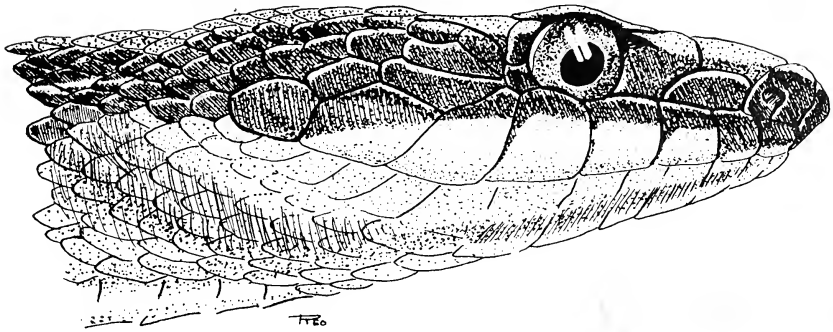

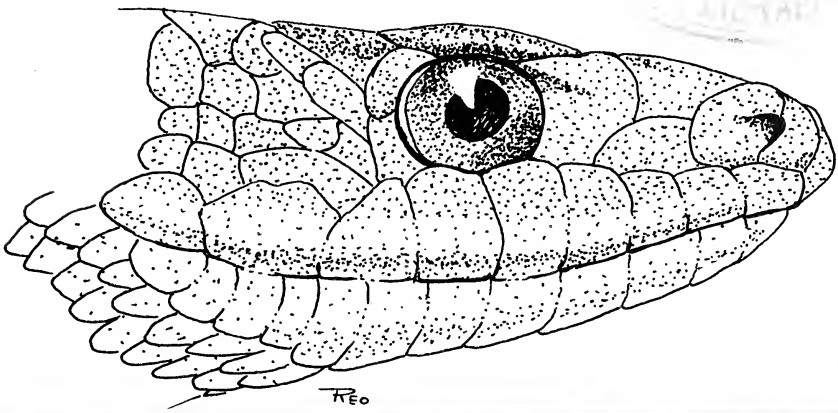

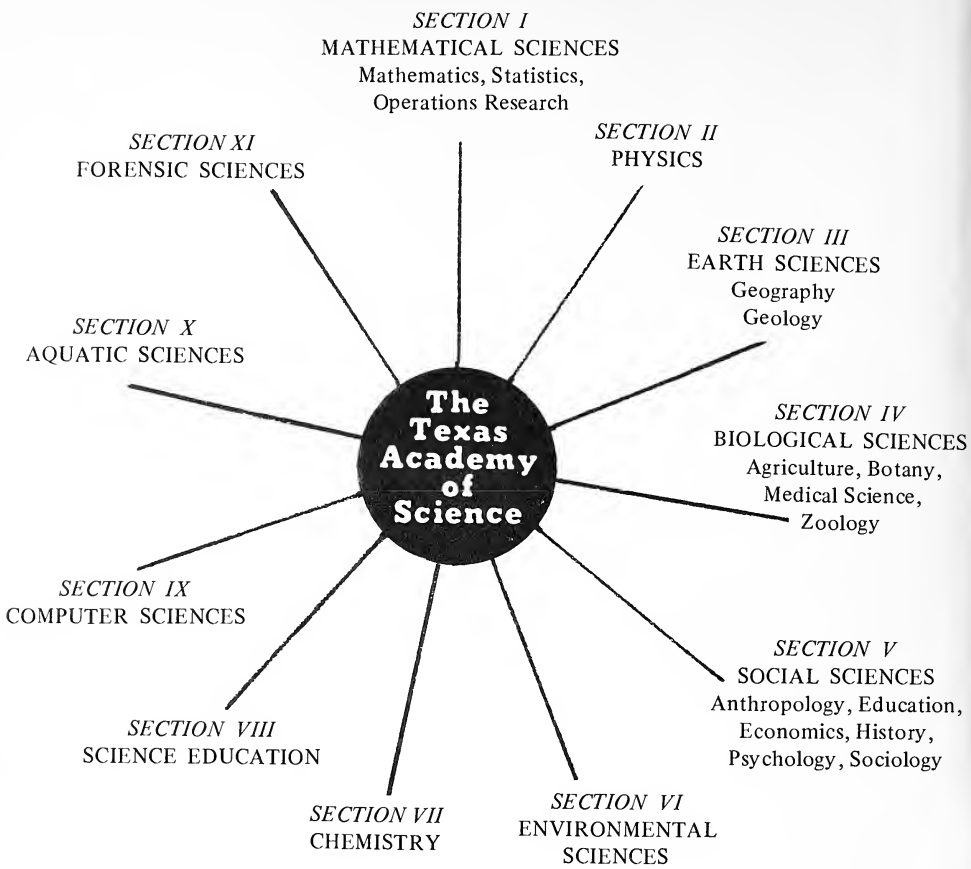


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# EFFECT OF BALCONES FAULTS ON GROUNDWATER MOVEMENT, SOUTH CENTRAL TEXAS

*by* PATRICK L. ABBOTT

*Department of Geological Sciences,*
*San Diego State University,*
*San Diego, California 92182*

ABSTRACT

The subparallel normal faults of the Balcones system have created an extensive fracture system in Lower Cretaceous limestones in south central Texas. The permeability avenues provided by the fractures have allowed surface water to enter already porous limestone and create extensive cavern systems that are especially pronounced in the Edwards Group. Some of the elongate, fault-bounded blocks of carbonate rock contain porosity systems that are partly independent of those in adjoining fault blocks. The channelization of groundwater flow within some fault blocks is indicated by preferential concentration of sinkholes, cave plans, and separate but parallel spring systems.

## INTRODUCTION

Heterogeneous dissolution of Lower Cretaceous limestones (Aptian to Albian) has created an extensive network of caverns in south central Texas. The Edwards Group has been most affected as has, to a lesser extent, the subjacent Walnut and Glen Rose Formations. These limestone bodies have minimal thicknesses that total several hundred feet and they extend over several thousand square miles, yet the significant development of caverns occurs mainly within the Balcones Fault zone (Fig. 1).

The carbonate rocks were formed with high initial porosities. During an interval of subaerial exposure at the close of Edwards Group deposition, some caverns were created. Nonetheless, the modern cavern system is primarily due to dissolution associated with faults. The Early Miocene subparallel, down-to-the-coast, normal faults of the Balcones system improved the access of surface water into the void systems of the subsurface rocks, but more importantly, the fault-rejuvenated erosion exposed discharge sites that allowed initiation of a continuously circulating groundwater system (Abbott, 1975). Groundwater undersaturated with respect to calcite and dolomite dissolves the carbonate rocks it flows through. Thus a self-ramifying process is set in motion whereby groundwater continuously enlarges its initial flow paths, thereby receiving ever-increasing amounts of groundwater. The original fault-created porosity and permeability have been greatly increased through time.

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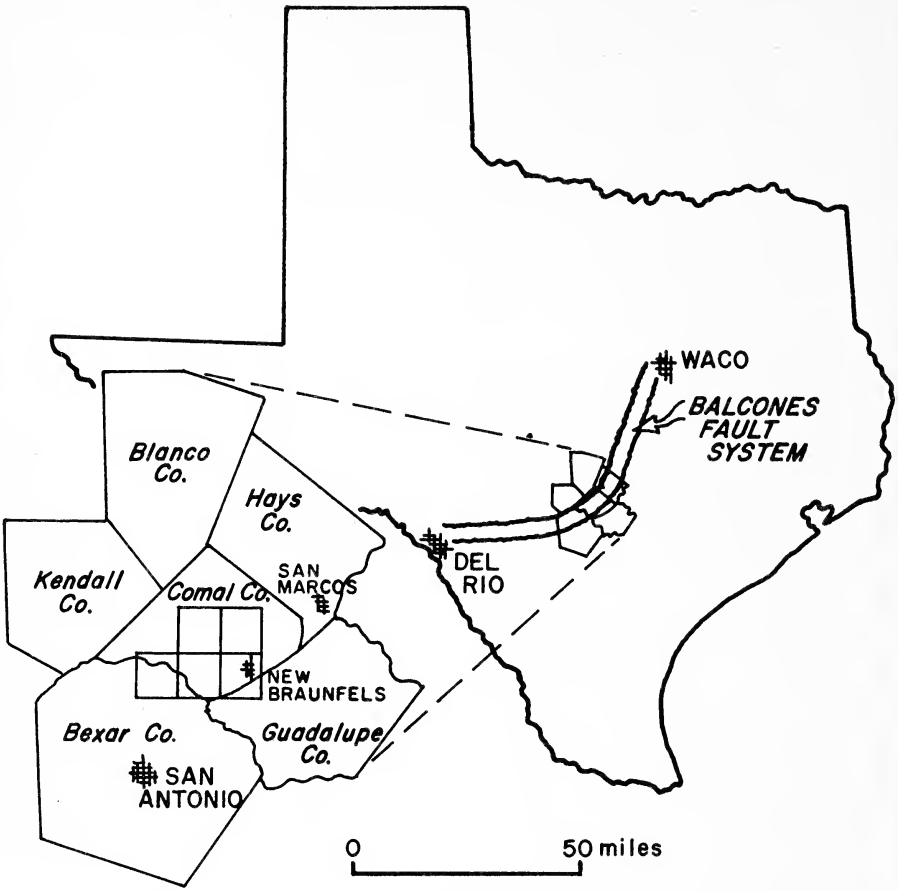


Figure 1. Location map. Reading counterclockwise from the lower left, the 7.5 quadrangles outlined in Comal County are: Bulverde, Bat Cave, New Braunfels West, Sattler and Smithson Valley.

A broad-scale understanding of the genetic relationship between the Balcones Faults and the extensive artesian aquifer has been well explained by Sayre and Bennett (1942) and discussed effectively by Hill and Vaughan (1898), Livingston, *et al.*, (1936), George (1948, 1952) and other workers. The purpose of this study was to examine the effects of individual faults on groundwater flow in order to better understand the limestone aquifers in Comal and Bexar Counties.

## LOCAL RELATIONSHIPS BETWEEN FAULTS, GROUNDWATER FLOW AND CAVERN DEVELOPMENT

There are several questions that might be raised about the role of the faults in affecting the flow of groundwater and thus the development of caverns. Have the

faults acted as conduits for lateral movement of groundwater along strike and, as a consequence, been enlarged into caverns? Have the faults acted as barriers that impeded the flow of groundwater by having juxtaposed impermeable rock masses against updip aquifer intervals? Have the faults placed different aquifer horizons together so that groundwater moves downdip across faults without noticeable hindrance? Or have all of the above occurred in different local areas? Several lines of evidence are developed that bear on these questions.

*Evidence From Plots of Groundwater Levels*

One way to evaluate the effect of faults on groundwater is to examine water levels in wells to see if marked differences exist on respective sides of a fault that would indicate a damming or compartmentalization. Conversely, the water-level elevations may present an evenly-sloping surface that apparently is unaffected by the presence of faults.

Several books of water-level elevations in wells have been published by the U.S. Geological Survey and the Texas Water Development Board. The data consist primarily of multiple readings on wells in the San Antonio and confined aquifer areas or of one-time water-level readings in the unconfined aquifer northwest of the main fault-line scarp of the Balcones system. Although many wells are available for mapping the groundwater flow pattern, the right kind of data is difficult to obtain. There are insufficient continuous hydrograph records, and it is impossible to utilize the typical water-level readings which were taken in widely varying months and years and thus reflect monthly and yearly fluctuations in rainfall. However, it is apparent from the water-level data in George (1952), Follett (1956), and Pettit and George (1956) that a field program was undertaken in May of 1945 to record the levels of as many wells as possible in as short a time as possible. Although the water levels in general declined during May, 1945, these records afford us as close an approximation to a map of the potentiometric surface as is available (Fig. 2).

The water level elevations plotted in Figure 2 are too scattered to allow definitive pronouncements about local groundwater movements. There is an overall slope of the potentiometric surface toward the southeast, but this is also the direction of the regional dip. An imperfect secondary trend of declining water-level elevations to the northeast within fault blocks is also discernible.

The wells north of the Waco Springs Fault draw water primarily from the Glen Rose Formation and those to the south from the Edwards Group. The potentiometric surface has a steeper slope north of the Bat Cave Fault which suggests that the cavernous porosity networks are not as well developed in the Glen Rose Formation. The few water levels available near the Bat Cave Fault do not indicate a barrier effect. Yet the Natural Bridge Caverns are developed on the upthrown block and abut directly against this fault (Jan Knox and Charles M. Woodruff, Jr., personal comm.).

Analysis of the effect of the Waco Springs fault suffers from a similar paucity of data points. The slope of the potentiometric surface in the block between the

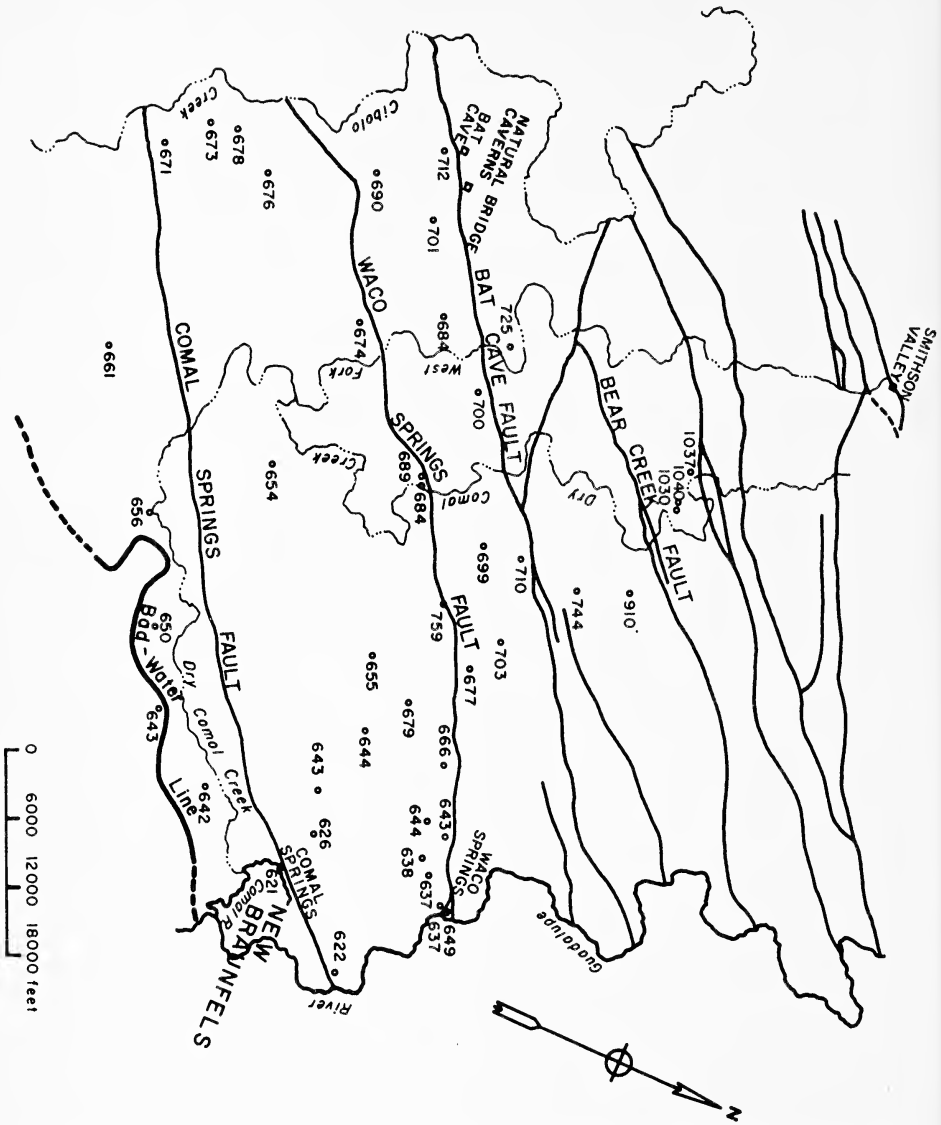


Figure 2. Structural and hydrologic map for a portion of Comal County. Well locations plotted as small circles; water levels recorded in May, 1945.

Waco Springs and Bat Cave Faults is steeper than in the blocks south of the Waco Springs Fault. An anomalously high value occurs at Krueger ranch (spot elevation 759 on Figure 2 just south of the Waco Springs Fault about 1 1/2 mi east of Dry Comal Creek) where large-scale cavern collapses have disrupted the strata. Note that water emerges from West Waco Spring at 649 ft elevation which is about 12 ft higher than the downdip water level. This suggests that the Waco Springs Fault has acted as a dam that concentrated groundwater along the fault.

Comal Springs arise along a 500-yd length of the Comal Springs Fault. At the springs, the throw along the fault has entirely displaced the Edwards Group and caused it to abut a relatively impermeable seal of Washita Division and Gulf Series strata. Southwest along the Comal Springs fault the throw decreases to about 200 ft at the southern boundary of the Bat Cave quadrangle (Newcomb, 1971). Groundwater apparently crosses the Comal Springs Fault easily in the southwestern part of the county where the Edwards is only partially offset.

Notice that the bad-water line pays no heed to the nearby presence of the largest fault in the Balcones Fault zone (Fig. 2). The bad-water line is a distinct boundary between the potable water of the Edwards artesian aquifer and the nonpotable water immediately downdip. The irregularity of this demarcation line and its disregard for faults suggest that it is the dissolution-ingrained flow boundary of the early groundwater that flowed to the initial discharge sites (Abbott, 1975).

*Evidence From Cave Maps*

The relationship of cavern formation to faults can probably best be visualized by inspection of caves. Two large caves (Bracken Bat Cave and Natural Bridge Caverns) developed near the Bat Cave Fault occur in the report area (Fig. 2). Maps of these caverns have been published by Reddell (1964) and adapted plans are shown in Fig. 3.

Both caverns have developed in the Glen Rose Formation on the upthrown block next to the Bat Cave Fault. The long segments of the caves are preferentially developed in near-horizontal fashion along bedding. In one stretch of Natural Bridge Caverns there are 2 subhorizontal passages developed at different levels within the 120-ft thick, upper dolomite subdivision of the Glen Rose Formation (Abbott, 1973). The different levels of cave formation in the Glen Rose Formation occur within a thick unit of relatively homogeneous lithology above the "alternating beds" interval. These interconnected, subhorizontal cavern passages probably make a good analogue to conditions within the generally similar rock types that comprise the Edwards Group to the southeast.

The trend of both cavern systems is largely perpendicular to and terminated near the Bat Cave Fault. The trend of the fault is roughly N60E and Bracken Bat Cave has formed along a N30W course. The right-angle orientation of the cave relative to the trend of the fault may have been inherited from a joint system formed at the time of the faulting.

The longest passages of Natural Bridge Caverns run approximately N30W, parallel to the trend of Bat Cave, and at right angles to the fault. Other large cave segments have developed along subparallel trends of about N30E. A lesser cavern branch has developed near to and parallel with the Bat Cave fault on a N60E course. The repeated subparallel cave passage trends suggest dissolution along fault-associated joint systems. Both caverns are developed on the updip side of the fault and seem to indicate that groundwater did not easily flow across that segment of the fault.

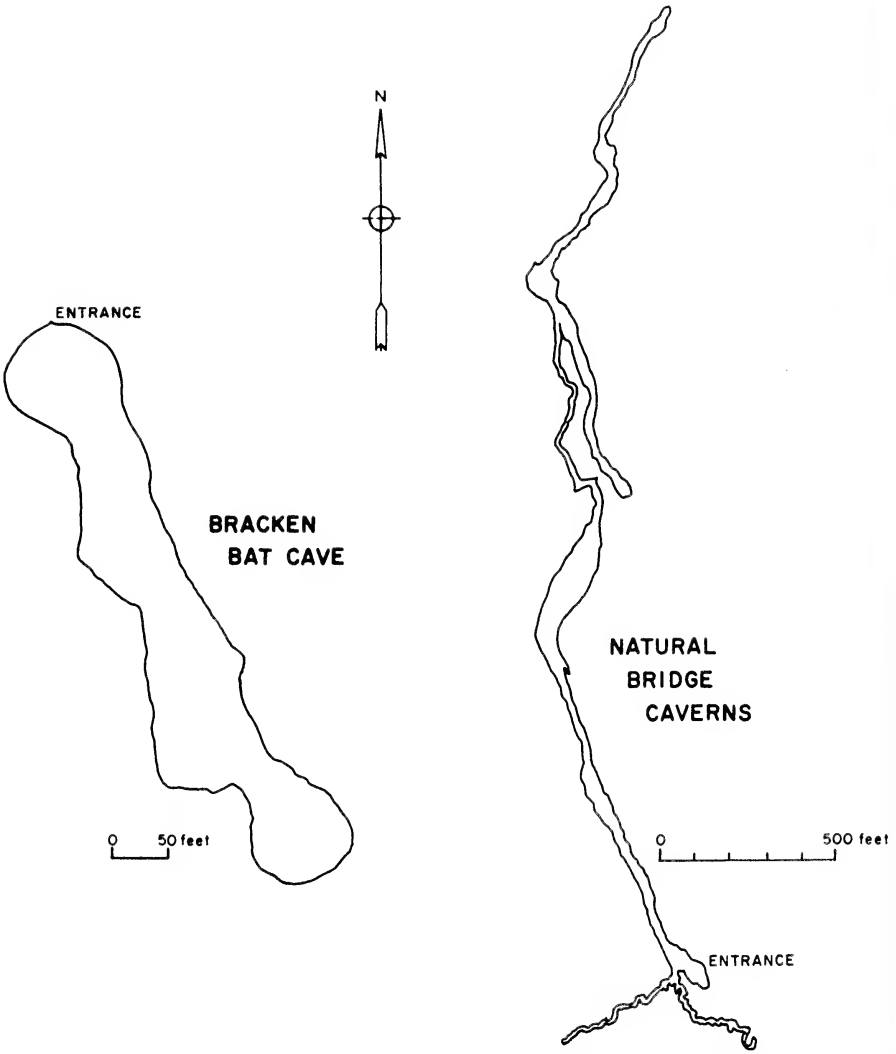


Figure 3. Maps of major passages of Bracken Bat Cave and Natural Bridge Caverns adapted from Reddell (1964). Cavern locations are shown in Fig. 2.

*Evidence From Preferential Development of Sinkholes*

The fault block between the Bat Cave and Waco Springs faults contains a profusion of sinkholes and some mega-collapse areas where beds have been chaotically deformed by the collapse of large caverns in regions of about 2 $mi^2$ each. The recognition of abundant karstic features in this fault block is partly a function of the surface stratigraphy but is probably also due to some groundwater channelization within the fault block.

The outcrops on this fault block commonly include a 22-ft thickness of clayey biomicrites of the Regional Dense Member which occurs about midway through the Edwards Limestone. The Edwards Group has been divided by Rose (1972) into the Kainer (lower) and Person Formations by separation at the base of the Regional Dense Member which is the basal unit of the Person Formation. Although the Regional Dense Member does not create an impassable seal, it seems to create locally perched groundwater bodies which flow laterally through the overlying strata. This process explains the profuse sinkholes, but the mega-collapse areas seem too large to be explained solely by this mechanism.

A mega-collapse area has been mapped in the northern part of the fault block on the former John Classen ranch that lies astride the Bulverde and Bat Cave quadrangles (Abbott, 1973). Here erratically dipping, petrified wood-bearing beds of the Person Formation (upper Edwards) have collapsed down through the Regional Dense Member into the Kainer Formation (lower Edwards). Prominent caverns still exist in this area.

An even more striking mega-collapse area occurs in a relatively narrow part of the Bat Cave-Waco Springs fault block on the Dietz Ranch in the northwestern part of the New Braunfels West quadrangle (Abbott, 1973). The ground surface is largely a mesquite-covered, cherty, terra rosa regolith which tends to obscure the detailed relationships of the collapses. However, it is clear that beds of the Georgetown and Del Rio Formations that overlie the Edwards Group are found at the general stratigraphic level of the Regional Dense Member and are completely surrounded by Edwards strata. This stratigraphic association, accompanied by some steep dips, suggests a downward collapse of about 200 ft. The abundant collapses in this 2 1/2 by 1 1/4 mi area may have followed the increased dissolution accomplished by groundwater forced to move through a narrower part of the fault block.

No evidence of similar mega-collapse features on adjacent and nearby fault blocks has been demonstrated to date. Thus, their presence in the Bat Cave-Waco Springs Fault block may be attributed partly to the long-term flow of groundwater confined largely to this fault block.

*Evidence From Spring Flow*

Waco and Comal Springs are major outlets that drain a large part of Comal and Comal/Bexar Counties, respectively (Fig. 2). They appear to be independent and unconnected systems although they are separated by only 3 mi in a north-south direction. These separate but parallel delivery systems strongly suggest that faults have aided the channelization of groundwater flow.

*Waco Springs.* The Waco Springs rise along the Waco Springs Fault at 2 sites in the floodplain just west of the Guadalupe River in the southeastern part of the Sattler quadrangle. Water emerges from outlets about 5 and 10 ft above the level of the Guadalupe River, at flows ranging from 0 to 96 $ft^3$/sec, with temperatures fluctuating between 68 and 71 F, and with turbid flow after heavy rains.

The Waco Springs Fault, and the dipping beds near it, appear to have had an impeding or channelizing effect on groundwater flow that has been responsible, in part, for the location of Waco Springs. Near the Guadalupe River the Waco Springs Fault splits into a sliver (Bills, 1957) from which the Waco Springs emerge out of arched beds that dip back to the northwest. This roll-over, plus the possible juxtaposition of less permeable beds on the downthrown side against more permeable beds on the upthrown side, has caused groundwater to be diverted along the fault. This diversion has been reinforced by development of a cavern system parallel to the fault which has grown in dominance through time.

The Waco Springs recharge and delivery system was partly explained by Bills (1957) who followed up the disposition of a localized August rainstorm during the drought of 1956 when the springs were not flowing. An isolated thunder storm dumped up to 5 in of rain in the vicinity of Smithson Valley (Fig. 2). The surface runoff went bank full down the west fork of Dry Comal Creek until reaching the Bear Creek Fault where it went underground. Approximately 24 hrs after this rain, the West Waco Spring began discharging about 2 $ft^3$/sec of slightly muddy water which subsided and ceased 2 days later.

The floodwater that went underground on the west fork of Dry Comal Creek at the crossing with the Bear Creek Fault apparently descended through the alluvial cover and the Walnut Formation into the upper dolomite subdivision of the Glen Rose Formation. The groundwater possibly moved toward the Bat Cave fault through a cavern system similar to the nearby Bat Cave and Natural Bridge Caverns. The groundwater apparently crossed the Bat Cave fault which juxtaposes the cavernous upper dolomite of the Glen Rose against the Kainer Formation (lower Edwards). Then the groundwater may have moved more or less diagonally across the narrow fault block between the Bat Cave and Waco Springs Faults, passed beneath the Dietz Ranch mega-collapse area, and emerged through contorted beds within a fault sliver along the Waco Springs Fault to flow out of West Waco Spring. Of course the floodwater that flowed down Dry Comal Creek may not be the same water which came out of West Waco Spring. Local flood recharge increased the head within the aquifer and the transmitted effect of the increased head may have caused the spring to flow.

The Waco Springs water-supply system apparently runs in large part to the east and northeast, or approximately at right angles to the surface drainage. The direction of flow seems to be subparallel to the faults and illustrates the effect of the faults on the groundwater flow.

*Comal Springs.* The flow of Comal Springs has varied between 0 to 420 $ft^3$/sec. It is one of the largest springs in the U.S. but the current and future discharge is lessened by the voluminous extractions of groundwater from wells in the San Antonio area. The average discharge is greater than the runoff from the 1,432 $mi^2$ drainage area of the Guadalupe River above the springs. The groundwater emerges in New Braunfels along a 500-yd length of the main fault-line scarp of the Balcones system (Comal Springs Fault) about a mile from the Guadalupe River, which is 40 ft

lower. The water is never turbid and its temperature remains steady at 74 F which is 6° higher than the average annual temperature at New Braunfels. This increased temperature has been accounted for by circulation paths between 300 to 500 ft below the surface (George, 1948, 1952).

The immense discharge of clear, constant temperature water indicates that Comal Springs is the outlet for groundwater collected over a vast area. To the north lies the effluent Guadalupe River and the isolated Waco Springs system which draws from the northwest. To the east and south is the highly mineralized water across the bad-water line. Thus the groundwater must be derived from the southwest. This means the subsurface water flow is subparallel to the Balcones Faults, about at right angles to the surface drainage, and at oblique angles to the regional dip and to the apparent regional piezometric surface contours. A master conduit system, or a greater volume of honeycombed and cavernous beds, has formed subparallel to the fault beneath a cover of younger rocks in analogous and parallel fashion to the Waco Springs system.

## CONCLUSIONS

Mapped caves trend at right angles to faults, mega-collapse regions are developed in certain fault blocks rather than along faults, and spring-supply systems commonly cross faults. These are evidence that fault surfaces are not the major sites of cavern development and that the various fault-defined blocks have had different degrees of cavern formation. The larger faults in Comal County (i.e., Comal Springs and Waco Springs Faults, and to a lesser degree the Bat Cave Fault) have acted in part as barriers that have diverted groundwater flow to the northeast. The location of most major cavern systems was determined largely by the porosity and permeability, plus structural controls incident to Balcones faulting, created by the early groundwater flow patterns established millions of years ago (Abbott, 1975). Thus the modern cavern locations are largely inherited and may display no easily discernible relationship to modern topography and stream courses.

## ACKNOWLEDGEMENTS

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# BAKING OF SHALE BY A BASALTIC DIKE: A CHEMICAL AND STRONTIUM ISOTOPIC STUDY

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ABSTRACT

Baking of friable, calcareous shale by approximately 1 m-thick alkalic basalt dikes in Trans-Pecos Texas resulted in prominent but relatively thin selvages of densely lithified shale adjacent to these dikes. Analyses of a sequence of baked and unaltered shale samples from one of the outcrops shows an infiltration of Fe, Mn, Mg, and possibly Sr into the host shale. The effects of baking of the shale, reflected in the clay mineralogy, also are shown by variations in the Sr isotopic compositions of whole-rocks, acid-leached residues, and acid supernates from samples of shale successively closer to the dike contact. Thermal effects by the intrusion apparently caused an approach to equilibrium of Sr isotopes between the marine carbonate and aluminosilicate fractions of the shale, as a function of increasing heat from the dike. However, complete homogenization was not achieved in a shale sample less than 1 cm from the dike contact. Thus, internal homogenization of Sr isotopes in detrital sediments is not achieved even at the relatively high temperatures ($\sim$ 1000 C) found near hypabyssal intrusions.

## INTRODUCTION

Although baking (mild, contact metamorphic effects resulting from hypabyssal intrusions and lava flows) is a common geologic feature, there are few chemical studies of the interaction between the magmatic and baked phases. In this study we report and discuss certain chemical and mineralogic features of calcareous shale baked by a basaltic dike.

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Baked tuff, conglomerate, and especially shale are prominent features in the Rim Rock dike swarm of Trans-Pecos Texas (Dasch, 1959; Dasch, *et al.*, 1969). The alkalic basaltic dikes, which range in age from about 18 to 23 m.y. (Dasch, *et al.*, 1969), transect Upper Cretaceous (Gulfian) calcareous shale and sandstone, and Tertiary volcanic strata (Vieja Group), mainly tuff, tuffaceous sedimentary rock, ignimbrite, and silicic flowrock. The approximately 100 dikes that have been mapped range in thickness from several cm to several m, but the majority are close to about 1 m in thickness. Baked host rock selvages range in thickness according to dike thickness; in shale exposures, the average, 1 m-thick dike has baked zones about 35 cm thick. Contacts between baked and unbaked shale are surprisingly sharp in most outcrops. In many exposures, baked rocks are more resistant to weathering than the basalt and stand as prominent parallel walls.

The yellow-weathering, calcareous shale adjacent to the dikes is densely lithified and somewhat darkened as a result of baking. Some thin sections show euhedral crystals of feldspar, pyroxene, and amphibole which apparently formed by infiltration of magmatic fluids into the shale. The dense, baked shale exhibits well developed cooling joints in most outcrops.

The dike selected for study (Dasch, *et al.*, 1969) has been analyzed in some detail (Dasch, 1969; Dasch, *et al.*, 1969). It is an alkalic basalt porphyry with large, partly resorbed phenocrysts of anorthoclase, kaersutite, biotite, plagioclase, and apatite. Gas vesicles in this amygdaloidal rock are filled with analcime and calcite. The fine-grained matrix consists of plagioclase, pyroxene, and scattered olivine crystals, with patches of poorly crystallized alkalic feldspar and analcime. The secondary minerals apparently result more from late-stage magmatic, or deuteric, alteration rather than from weathering (Dasch, 1969).

## METHODS

Samples were obtained from the dike (about 12 cm into the dike from the dike-shale contact), from baked shale 2.5 cm (S-5) and 23 cm (S-8) from the contact, and from apparently unmetamorphosed shale 183 cm (S-10) and 610 cm (S-11) from the contact.

Carbon was determined at Oregon State University on water-rinsed samples utilizing a Leco carbon analyzer. Rock powders for the other major elements were ignited at 1000 C for several hours to liberate $H_2O$ and $CO_2$, and to oxidize the Fe. Excepting Sample S-10, element concentrations then were determined by X-ray fluorescence spectrometry at the Manned Spacecraft Center (NASA), on a glass disc prepared by fusing a 280 mg aliquant of the sample with a lanthanum-bearing, lithium borate fusion mixture (Norrish and Hutton, 1969). Calibrations were based on primary synthetic standards supplemented by previously analyzed U.S.G.S. and N.B.S. rock and mineral standards. Na was analyzed by atomic absorption spectrometry on a separate 20 mg aliquant of the sample. All samples were analyzed in duplicate.

Sr was separated from the samples by standard techniques of dissolution and ion exchange. Sr isotope ratios were measured on rhenium triple filaments on the 12-in radius of curvature, 60° sector mass spectrometer at Lamont-Doherty Geological Observatory. Precision on $Sr^{87}/Sr^{86}$ ratios = ± 0.0002 except as noted in Table 2. $Sr^{87}/Sr^{86}$ values were normalized to a $Sr^{88}/Sr^{86}$ value of 8.3752.

## RESULTS AND DISCUSSION

As with other dikes in the Rim Rock dike swarm (Dasch, 1959) the analysis of Dike 188 (Table 1) shows it to be a soda-rich, alkalic basalt; the large amount of apparently primary analcime indicates a composition approaching phonolite. Calcite from one of the large, calcite-analcime amygdales was analyzed by G. D. Garlick (personal communication) for O and C isotopic composition. The results, $\delta O^{18}$ = +17.6°/oo(SMOW), $\delta C^{13}$ = -3.8°/oo(PDB), indicate that the calcite is clearly secondary. Textural relations in matrix analcime, however, suggest that much of this feldspathoid is deuteric in origin. A calcite amygdale from this dike has a $Sr^{87}/Sr^{86}$ ratio of 0.7056, whereas the dike itself is much less radiogenic, 0.7041 (Dasch, 1969); Sr from the secondary calcite thus is at least partly derived from sources other than the original magma--such as sedimentary calcite from the adjacent shale.

TABLE 1

Major element concentrations of Dike Rock 188, and unbaked (S-11) and baked (S-5, S-8) samples of shale.

| | 188 (interior of dike) | S-5 (baked shale, 2.5 cm from contact) | S-8 (baked shale, 23 cm from contact) | S-11 (unaltered shale, 610 cm from contact) |
|---|---|---|---|---|
| $SiO_2$ | 47.11 | 48.26 | 55.93 | 48.10 |
| $TiO_2$ | 2.28 | 0.65 | 0.56 | 0.64 |
| $Al_2O_3$ | 16.76 | 15.21 | 12.48 | 14.51 |
| $Fe_2O_3$ | 10.28 | 6.54 | 4.28 | 4.82 |
| MnO | 0.13 | 0.09 | 0.06 | 0.02 |
| MgO | 4.88 | 2.91 | 1.84 | 1.93 |
| CaO | 8.35 | 17.34 | 17.03 | 18.43 |
| $Na_2O$ | 4.79 | 4.97 | 4.29 | 3.17 |
| $K_2O$ | 2.76 | 0.86 | 0.64 | 2.09 |
| $P_2O_5$ | 1.43 | 0.19 | 0.18 | 0.16 |
| S | 0.10 | 0.03 | 0.02 | 1.57 |
| C* | 0.91 | 3.37 | 3.63 | 3.20 |
| Totals | 99.78 | 100.42 | 100.94 | 98.64 |

*Total C measured prior to ignition.

Apparently unmetamorphosed shale (S-10, S-11) and progressively more intensely baked shale (S-8, S-5) have compositions as shown in Table 1. Initial chemical variations within this detrital unit undoubtedly mask many minor changes brought about by baking. However, the data suggest rather marked increases in Fe, Mn, and Mg as a result of the contact effects. As euhedral, apparently igneous minerals are apparent in thin sections of the more highly baked rocks, it is obvious that some infiltration of magmatic fluid has occurred.

Mineralogic analysis of whole-rock samples of S-11, S-8, and S-5 were made by E. A. Perry (personal communication). Sample S-11 is mainly composed of a mixed-layer illite/smectite with about 20% smectite and ordered (allevardite-type) interlayering. It also contains about 15 to 20% chlorite and kaolinite and appears to be completely unmetamorphosed. Samples S-8, from the baked zone, contains no mixed-layer clay, illite, or potassium mica; it is comprised largely of chlorite, quartz, low albite, and potassium feldspar. Sample S-5 is much like S-8, but the chlorite is better crystallized, and, along with feldspar, is somewhat more abundant than in S-8. The mineralogy of Samples S-8 and S-5 thus exhibit the effects of progressive contact metamorphism.

Strontium isotope analysis (Table 2; Figure 1) of the samples indicates that comparatively nonradiogenic Sr of the dike ($Sr^{87}/Sr^{86}$ = 0.7040) infiltrated the more radiogenic shale, lowering the $Sr^{87}/Sr^{86}$ ratios in whole-rock samples of the shale from about 0.709 to about 0.707 adjacent to the dike. Our interpretation of these data is consistent with other observations: the dike is a ready source of Sr (1425 ppm), and euhedral minerals occur in the baked rocks, which apparently formed as a result of fluid infiltration.

TABLE 2

Strontium isotopic composition of dike, and of whole-rock, acid supernate, and acid-leached residue from shale samples. $Sr^{87}/Sr^{86}$ ratios normalized to $Sr^{88}/Sr^{86}$ = 8.3752. Analytical uncertainties (2$\sigma$) on $Sr^{87}/Sr^{86}$ = ±0.0002 except for acid supernate for S-11 (±0.0003) and leached residue for S-8 (±0.0004).

| Sample Number | Distance From Dike-Shale Contact, cm | Whole Rock | $Sr^{87}/Sr^{86}$ Acid Supernate | Acid-leached Residue |
|---|---|---|---|---|
| Dike Rock (188) | 12.0 | 0.7040 | -- | -- |
| S-5 | 2.5 | 0.7075 | 0.7072 | 0.7083 |
| S-8 | 23.0 | 0.7076 | 0.7072 | 0.7086 |
| S-10 | 183.0 | 0.7084 | 0.7074 | 0.7104 |
| S-11 | 610.0 | 0.7090 | 0.7080 | 0.7122 |

Of more significance, Sr between carbonate and aluminosilicate fractions in the shale has become partially equilibrated as a function of increasing degree of baking. In addition to whole-rock samples, Sr isotope analyses of the shale were

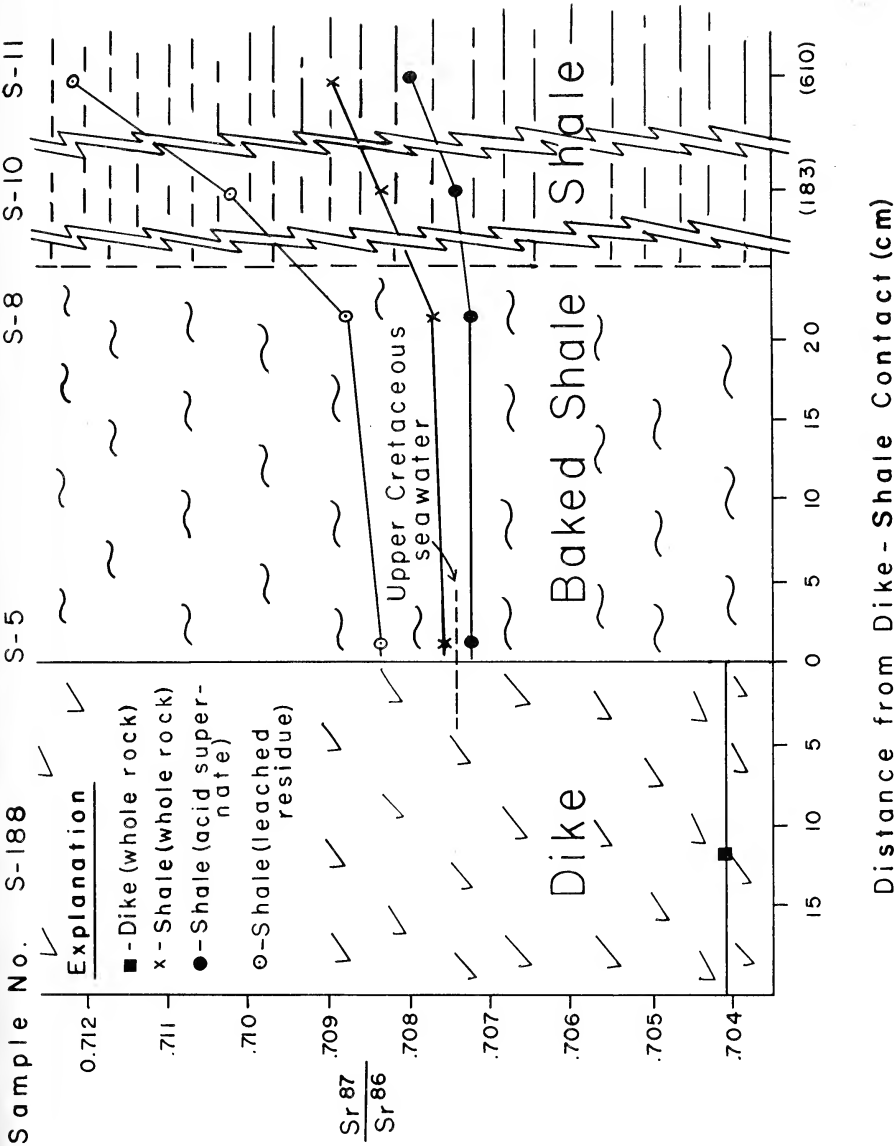


Figure 1. $Sr^{87}/Sr^{86}$ ratios of Dike Rock (188), and unbaked (S-11) and baked (S-5, S-8) samples of shale *vs.* distance from dike-shale contact. Except for the acid-leached residues of Samples S-10 and S-11, the measured ratios are the same as the ratios at the time of formation (initial) of the rocks. Initial ratios of the acid-leached residues of S-10 and S-11 are about 0.001 less than the measured values.

made on supernate and acid-leached sample aliquants. In all samples (Figure 1), Sr in the aluminosilicate residue is more radiogenic than Sr associated with the

leached carbonate. Carbonate (acid supernate) Sr of the Upper Cretaceous, unmetamorphosed shale (S-11) has an $Sr^{87}/Sr^{86}$ ratio of 0.7080, close to the value reported for Sr dissolved in Upper Cretaceous seawater (Peterman, *et al.*, 1970). However, in samples closer to the dike-shale contact, the spread between $Sr^{87}/Sr^{86}$ ratios of Sr in carbonate and Sr in aluminosilicate phases is reduced from about 4 parts in 700 to 1 part in 700, ostensibly by equilibration resulting from increasing metamorphism.

These data have some relevancy to the problem of dating shales by Rb-Sr techniques. Many geochronologists (eg. W. Compston *In* Bofinger and Compston, 1968) believe that isochronism in detrital sediments may be brought about by early Sr homogenization, owing to relatively mild burial metamorphism or diagenesis (see also Perry and Turekian, 1974). Our data show that homogenization was approached but not achieved, in the specific case of this baking study, but only at temperatures near 1000 C at the dike-shale contact. Of course, the baking process probably is short-lived; much longer periods of, say, burial metamorphism, even at much lower temperatures, probably is more effective as a homogenizing process.

ACKNOWLEDGEMENTS

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# INFLUENCE OF ANISOTROPIES ON THE SHEAR STRENGTH AND FIELD BEHAVIOR OF HEAVILY OVERCONSOLIDATED, PLASTIC AND EXPANSIVE CLAY-SHALES

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ABSTRACT

Heavily overconsolidated, highly plastic and expansive clay-shales crop out in the north central Texas region. These shales have given rise to problems related to engineering construction. Slope instability, high potential volume change, and low shear strength characterize these materials.

The highly anisotropic character of the clay-shales has been found to have a pronounced effect on their field behavior. Their fractured and fissured character provides them with preexisting planes of weakness which enhance slope instability and related problems. Field investigations and laboratory testing demonstrate that if these anisotropies are not taken into account, it is possible to greatly overestimate the shear strength of the shales, a fact that can lead to disastrous results.

## INTRODUCTION

The shales of the Taylor, Eagle Ford, Woodbine, and Washita groups of the Cretaceous System crop out in the vicinity of the Waco urban area in north central Texas. These shales have become well-known for giving rise to problems related to engineering construction. Detailed field studies and a rigorous program of laboratory testing have demonstrated that all of the above mentioned shales are highly anisotropic. These anisotropies are found to have a pronounced effect on their field behavior.

The purpose of this study is to document the effects of these anisotropies on the shear strength and field behavior of the Waco shales. It is expected that this knowledge may help prevent problems in the future urbanization of the region.

## LOCATION AND PREVIOUS WORK

The study area lies in and around the Waco urban area within McLennan County, Texas (Fig. 1).

In 1972, Wright and Duncan recognized the highly anisotropic character of the Pepper Shale in Waco (Table 1). The authors stated in their paper concerning the analysis of the Waco Dam slide, that the Pepper Formation of the Woodbine

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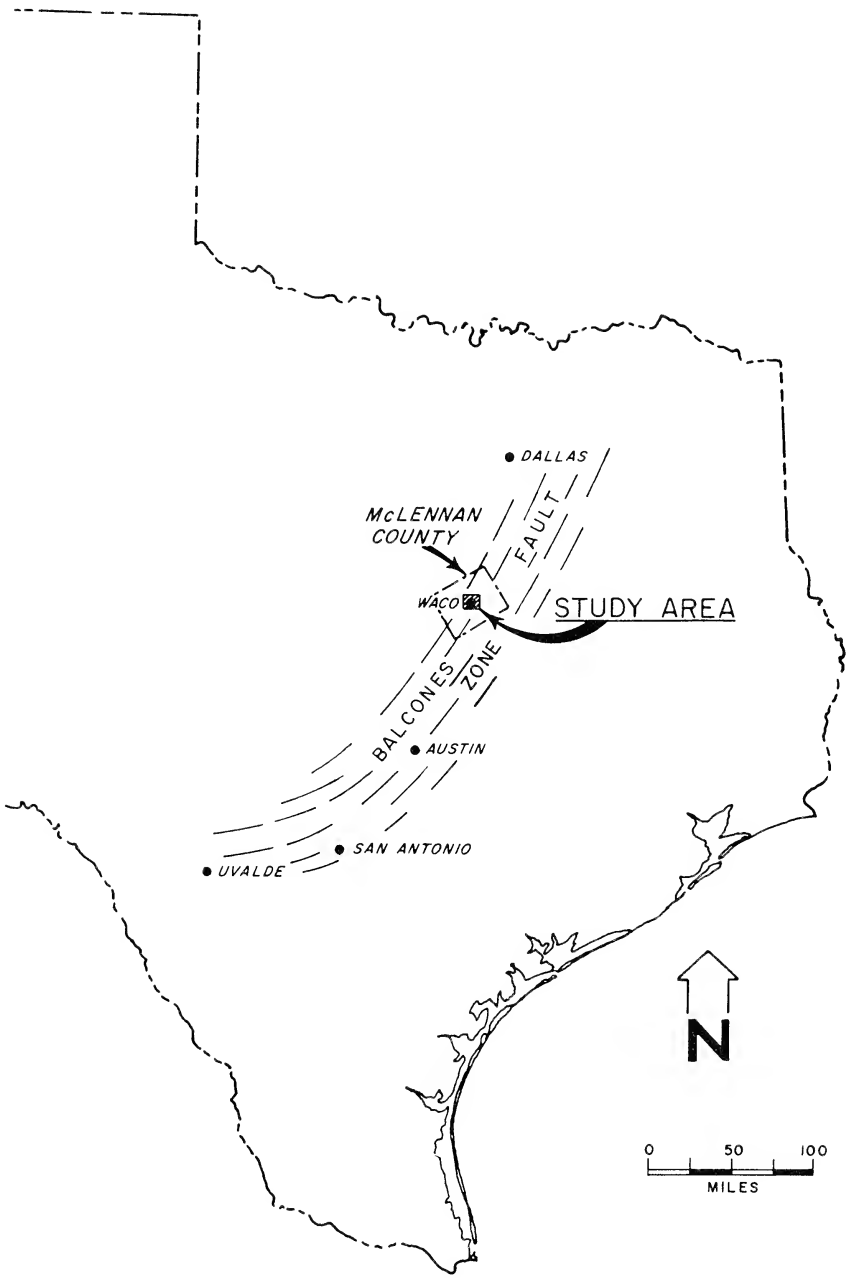


Figure 1. Location Map.

TABLE 1

Stratigraphic Relationship, Depositional History and Maximum Thickness of the Cretaceous Strata in the Local Area

| Series | Group | Formation and Symbol | Depositional History | Maximum Local Thickness in feet | Maximum Probable Thickness at one time in feet |
|---|---|---|---|---|---|
| Gulf | Taylor | Taylor Marl $K_{ta}$ | Deposited during marine transgressions and regressions. | 250 | 1170 |
| " | Austin | Austin Chalk $K_{au}$ | Deposited during marine transgression with possible fluctuations of the strandline Unconformably underlies the $K_{ta}$. | 250 | 295 |
| " | Eagle Ford | South Bosque Shale $K_{sb}$ | Deposited in a neritic marine environment with poor circulation. Unconformably underlies the $K_{au}$. | 160 | 160 |
| " | " | Lake Waco Fm. $K_{lw}$ | Deposited in a lagoonal environment. Conformably underlies the $K_{sb}$. | 80 | 145 |
| " | Woodbine | Pepper Shale $K_{pe}$ | Deposited in brackish environment. Unconformably underlies the $K_{lw}$. | 70 | 100 |
| Comanche | Washita | Buda Limestone $K_{bu}$ | Nearshore marine deposit. Almost completely eroded locally prior to deposition of $K_{pe}$. | 2 | 35 |
| " | " | Del Rio Clay $K_{dr}$ | Marine regression and transgression. Restricted environment. Conformably underlies the $K_{bu}$. | 85 | 85 |
| " | " | Georgetown Limestone $K_{ge}$ | Shallow marine deposit. | 210 | 210 |

Group is horizontally fissured. They also demonstrated that on the average, the strength along these fissured planes is 40% lower than the strength obtained by testing samples in a conventional style, so that the shear plane does not coincide with a preexisting plane of weakness. Wright and Duncan (1972), in fact, related the failure of the Waco Dam to the highly anisotropic character of the Pepper Shale.

Studies by Font from 1969 to 1976 have led to the conclusion that all of the Waco shales are highly anisotropic. Data supporting this conclusion are presented in this paper.

Previous works regarding the engineering geology of the Waco urban region include those of Burket (1965), Font (1969), Font and Williamson (1970), and Font (1973a, 1973b, 1974, 1975, 1976a, 1976b).

## GEOLOGIC SETTING

Most of the bedrock exposed within Waco include marine limestones and shales of Cretaceous age. The Waco region is transgressed by the Balcones Fault Zone, a zone of normal faults that cuts across Texas (Fig. 1). The Balcones Faults in Waco trend NNE and are commonly downthrown to the east. Maximum displacement along these faults is approximately 300 ft.

The stratigraphic relationship, depositional history and maximum thickness of the Cretaceous strata are summarized in Table 1.

### *Stress History*

The Waco shales are all heavily preloaded. Studies indicate that 500 to 2000 ft of overburden overlain the top of these shales at one time (Font, 1976a). Since overburden on top of these shales is now either totally absent or at the most only a few feet thick, the Waco shales are heavily overconsolidated. The field instability and stress history of the Waco shales is reflected by their engineering properties as indicated in Table 2.

## EFFECT OF ANISOTROPIES ON THE SHEAR STRENGTH AND FIELD BEHAVIOR OF THE WACO SHALES

In order to study the effect of anisotropies on the shear strength and field behavior of the Waco shales, a series of consolidated-undrained direct shear tests simulating field conditions have been conducted on remolded and undisturbed samples of these materials. Most of the tests have been performed in a model D-120[b] direct shear apparatus manufactured by Soil Test Inc. Sensitivity and unconfined compressive strength values have been obtained by running unconfined compression tests on undisturbed and remolded samples at the same water content. The apparatus used in these tests is a model U-560 manufactured also by Soil Test Inc. The results obtained for all of the Waco shales are similar. In view of this, results obtained for the Del Rio Formation of the Washita Group

TABLE 2

Index Properties of the Waco Shales.

| Liquid Limit | Plasticity Index | Potential Volume Change | Unified Soil System Category |
|---|---|---|---|
| 60 to 80 | 30 to 52 | Marginal to Very Critical | CH |
| | | | |
| Percent Clay | Percent Calcium Carbonate | Dry Unit Weight P C F | Liquidity Index |
| 75 to 95 | 2 to 30 | 97 | Zero to Negative |
| | | | |
| Sensitivity | | Residual Strength Parameters | |
| 1.0 to 1.2 | | $\overline{\emptyset}_r = 5° - 10°$ <br> $\overline{C}_r = 0$ | |

are the only ones presented here. The results obtained for the shales of the Taylor, Eagle Ford, and Woodbine groups have been found to be nearly identical, but even more pronounced than those obtained for the Del Rio Formation.

Figure 2 depicts 3 Mohr rupture envelopes obtained in testing the Del Rio Formation. The upper rupture envelope has been obtained by testing undisturbed samples, where the shear plane does not coincide with a preexisting plane of weakness or anisotropic plane. The middle envelope depicts the results obtained by testing normally consolidated remolded samples. The lower envelope has been obtained by testing undisturbed samples of the Del Rio Formation, where the samples have been oriented, so as to insure that the shear plane developed in the test coincides with preexisting planes of weakness or planes of anisotropy. It can be seen that the strength along preexisting planes of weakness is drastically lower than that which could be expected by using the shear strength parameters of the upper envelope of Fig. 2. On the average, it has been found that the strength along preexisting planes of weakness is from 2.5 to 4 times lower than that expected from the upper Mohr envelope. The important point to establish now is which strength actually operates in the field. In order to accomplish this goal, several slope failures within Waco have been analyzed. Slope stability analyses have been performed on these slopes using the shear strength parameters shown in the upper and lower Mohr envelopes of Fig. 2. It should be recalled that if a slope is stable,

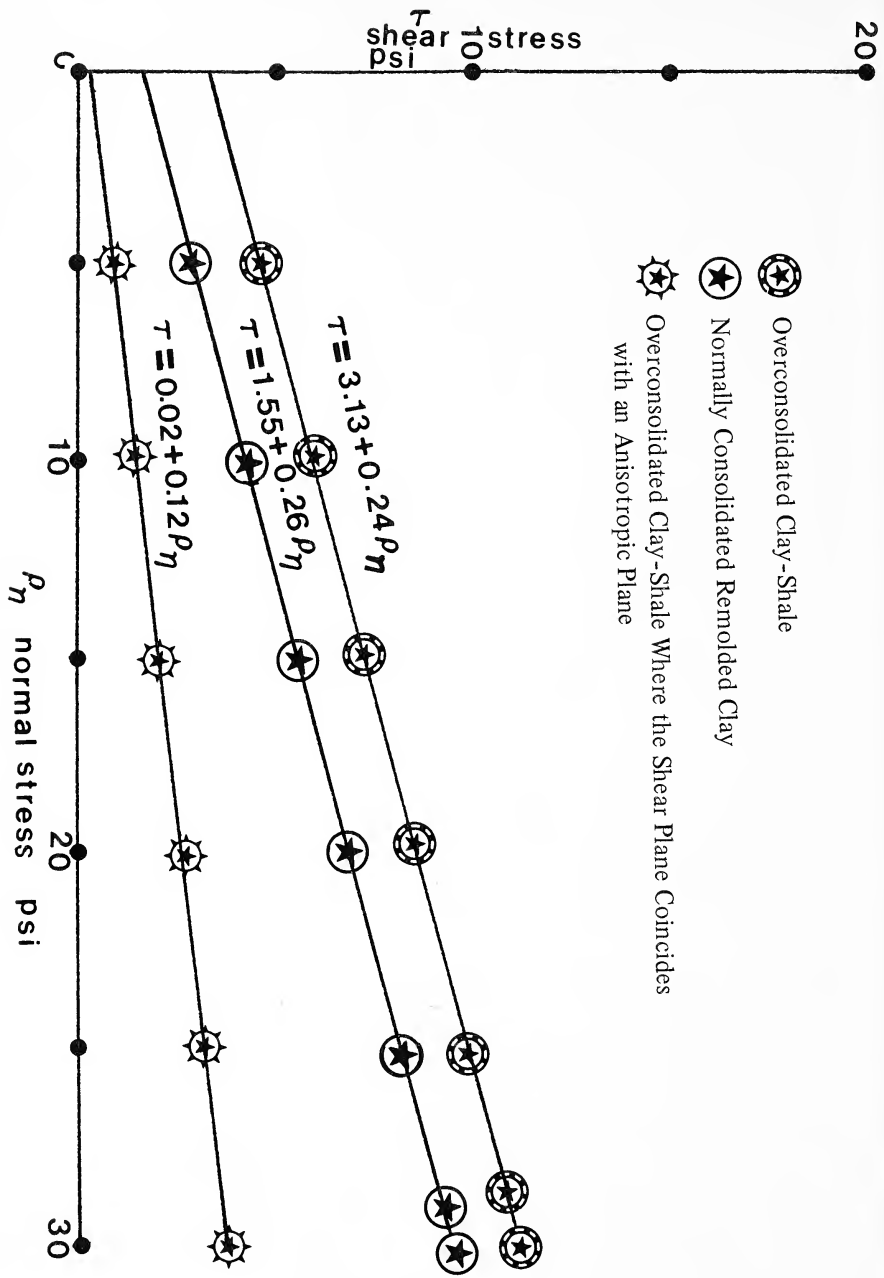


Figure 2. Mohr rupture envelopes for the Del Rio Formation.

the slope stability analysis must yield a factor of safety greater than 1.0. It should also be indicated that all of the slopes that have been analyzed by the author in this study had already failed. In using the shear strength parameters which define

the upper rupture envelope in Fig. 2, factors of safety considerably greater than 1.0 are obtained. This would indicate that the slopes are stable. However, since the slopes have already failed, this could not be true. The shear strength parameters that actually predict the instability of these slopes have been found to be those of the lower envelope of Fig. 2.

## CONCLUSIONS

It is found in analyzing several slope failures in the Waco shales, that the shear strength at failure for these materials is very close to that which coincides with the strength along preexisting planes of weakness. Thus, it is highly possible to greatly overestimate the field strength of these shales if these anisotropies are ignored. It is imperative to take into account the influence of these anisotropies in future engineering endeavors if we wish to prevent catastrophic failures, such as that of the Waco Dam.

## ACKNOWLEDGEMENTS

I would like to express my gratitude to Wayne Mudd and Patrick Millegan for their help in some of the laboratory testing. Special thanks are offered to Dr. G. A. Morales of Baylor University for reviewing this manuscript. I am thankful to Mrs. Viola Shivers for typing the manuscript.

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# SEX RATIOS AND SPAWNING OF WHITE BASS, *MORONE CHRYSOPS*, FROM THE RED AND WASHITA RIVER SEGMENTS OF LAKE TEXOMA[1]

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ABSTRACT

Sex ratios of white bass, *Morone chrysops*, from the Red and Washita River segments of Lake Texoma were studied with reference to seasonal variations. The gonadosomatic index was used to determine the duration of the spawning season.

## INTRODUCTION

Fish production in many reservoirs declines markedly after the first few years (Ellis, 1937). The white bass is a valuable species because it often maintains a sport fishery in older reservoirs as the initial high production of game fishes declines.

In Lake Texoma, a 36,423 ha impoundment of the Red and Washita Rivers in southern Oklahoma and northern Texas, the white bass is probably the most sought-after species. To formulate a rational management program, knowledge of its life history is necessary. This paper provides additional information on the sex ratios and spawning of this species in Lake Texoma.

## MATERIALS AND METHODS

From September 1969 to July 1972, 1,540 white bass were collected from the Red and Washita Rivers which form Lake Texoma, primarily using gill nets with 1.5-in to 2-in square mesh. An electroshocker mounted on a 14-ft flat-bottom boat with a 220V generator also was used. Seining and angling were other methods of capture.

Immature fish were sexed by removing a thin cross-section from one gonad, placing it on a glass slide with a drop or two of aceto-orcein, and examining with

[1]This is part of a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Oklahoma.

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a microscope. This technique allowed the detection of immature ova in the females. Mature fish could readily be identified by external examination. The duration of the spawning period was determined by studying the gonadosomatic index, defined as the ovary weight divided by the total body weight times 100.

## RESULTS AND DISCUSSION

Most Red River samples were taken approximately 24 km (15 mi) upstream from the lake, and most white bass were taken from the Washita River about 16 km or 10 mi from Texoma. On April 14, 1970, however, a collection of 8 males and 2 females was taken by electroshocking in Wild Horse Creek, a tributary of the Washita River approximately 161 km (100 mi) upstream from Texoma. Ranchers say they catch white bass there only in the spring.

Schooling by sex occurred during the white bass's migration. For example, on April 4, 1970, a mass migration passed through Cumberland Cut where the Washita River enters Lake Texoma. Of 86 fish collected, all were males. Unisexual schooling has also been reported for the white bass by Riggs (1955) in Lake Shafer and Lake Texoma.

The Red River samples show that for 6 mo out of 11, males outnumbered females; in the Washita River, males were more numerous than females 2 mo out of 7 (Table 1). However, significant deviation from 1:1 sex ratios occurred in Red River samples only for the month of March and significant deviation occurred in the Washita River only in February.

TABLE 1

Monthly Sex Ratios for White Bass from the Red and Washita River Segments of Lake Texoma for 1969-72.

| Location | Month | Number of White Bass | Sex Ratio (Females/Males) | df[1] | $x^2$[2] |
|---|---|---|---|---|---|
| Red River | | | | | |
| | January | 8 | 0.33 | 1 | 2.00 |
| | February | 182 | 0.80 | 1 | 2.20 |
| | March | 137 | 0.36 | 1 | 30.84[3] |
| | April | 51 | 1.43 | 1 | 1.58 |
| | May | 25 | 1.27 | 1 | 0.36 |
| | June | 18 | 0.80 | 1 | 0.22 |
| | July | 99 | 1.20 | 1 | 0.82 |
| | August | 6 | 0.50 | 1 | 0.66 |
| | September | 62 | 1.21 | 1 | 0.58 |
| | October | 24 | 1.40 | 1 | 0.66 |
| | November | 30 | 0.76 | 1 | 0.54 |
| Washita River | | | | | |
| | January | 65 | 1.03 | 1 | 0.02 |
| | February | 184 | 2.07 | 1 | 22.26[3] |
| | March | 87 | 0.89 | 1 | 0.28 |
| | April | 470 | 0.84 | 1 | 3.76 |
| | May | 23 | 1.30 | 1 | 0.40 |
| | June | 13 | 5.50 | 1 | 6.24 |
| | November | 56 | 2.73 | 1 | 2.70 |

[1] Degrees of freedom
[2] Chi-square values
[3] Significant at 0.01 level

Casselman (1975) found that sex ratios of northern pike, *Esox lucius,* from 3 Ontario populations showed seasonal trends in his samples. He believed that these peaks of availability were related to activity and were independent of locality and method of capture. The seasonal differences observed in white bass in this study may be related to sexual differences in prespawning activity.

The gonadosomatic index (Fig. 1) showed that the ovaries of white bass from Lake Texoma were most developed in March and April, and that by May they were mostly spent. The spawning season, therefore, probably started in late March and was completed by early May. Taber (1969), studying larval fish in the Buncombe Creek arm of Lake Texoma,gave the first of April to early May as the white bass's Spawning season.

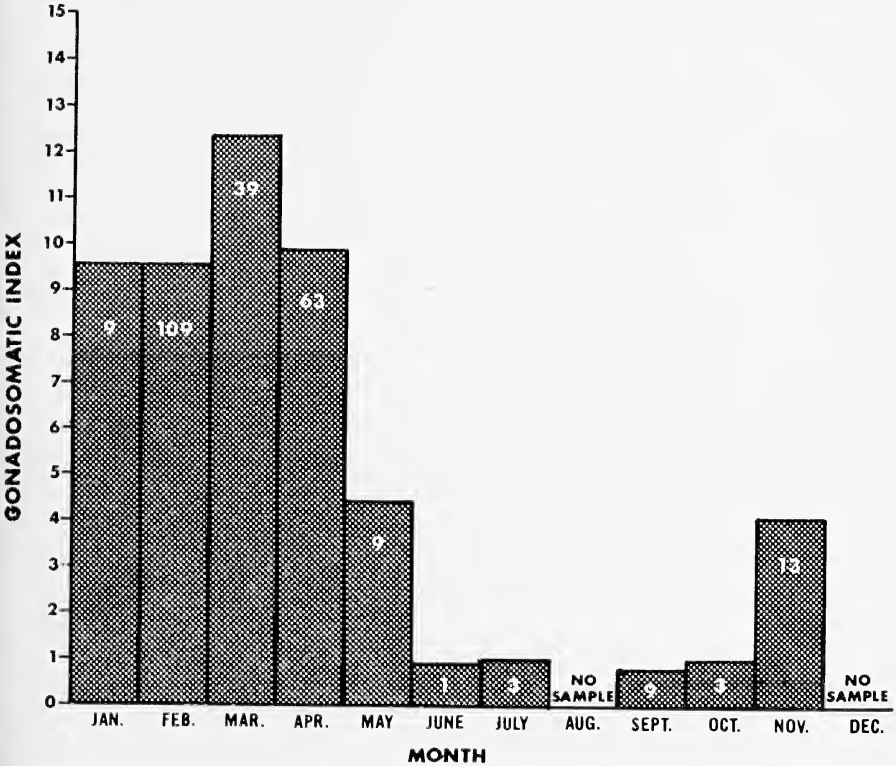


Figure 1. Seasonal variation in mean gonadosomatic index (ovary weight/body weight x 100) of Lake Texoma white bass for 1969-72. Numbers of fish are indicated.

ACKNOWLEDGEMENTS

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# DENSITY AND DISTRIBUTION OF THE BLACK-TAILED PRAIRIE DOG IN TEXAS[1]

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ABSTRACT

More than 30,000 Agricultural Stabilization and Conservation Service aerial photographs providing coverage in 108 counties in central and western Texas were examined to provide management data on the current population size and distribution of the black-tailed prairie dog (*Cynomys ludovicianus*). In areas of incomplete photographical coverage, individuals knowledgeable of black-tailed prairie dog colony locations were interviewed. A total of 1,336 prairie dog colonies inhabiting 36,432 ha were located in 89 counties in the study area. On-site inspections were made at 319 colonies (23.8% of total) to verify the colony's presence, size, and location. The average colony size was 27.27 ha. Croplands presently utilize 29.4% of the study area's prairie dog habitat. A total of 1,268 burrowing owls (*Speotyto cunicularia*) were observed in 91 of the 319 colonies inspected.

## INTRODUCTION

In literature describing the black-tailed prairie dog, the species' historic population, circa 1900, is frequently compared with the present population. The historic population in Texas is impressive and few authors dispute a considerable decline; but the degree of the decline is questionable and the present population has been difficult to ascertain. Control efforts are emphasized by many authors as the major factor involved in the population's decline while land use changes, if acknowledged, are not well described.

The historic prairie dog population in Texas was estimated by Bailey (1905) at 800,000,000 animals inhabiting 233,100 $km^2$. "Their range extends from Henrietta, Fort Belknap, Baird, and Mason west almost to the Rio Grande, north over the Staked Plains and the Panhandle region, and south to the draws of Devil's River, to 10 mi south of Marathon and 25 mi south of Marfa" (Fig. 2).

Cottam and Caroline (1965) provided some conservative estimates on the present population. They polled reliable individuals residing in counties containing prairie

[1]This management survey was suported and administered by the U.S. Fish and Wildlife Service, Division of Wildlife Services, cooperating with Texas A&M University Agricultural Extension Service.

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dogs and concluded that Texas contained a minimum of 300 colonies. Other authors have described a more tenuous abundance and some factors responsible for the population's decline.

Rue (1967) said, "Today, poison, traps, and guns have all but wiped out what was once one of our most common western animals." Drimmer (1954) reported, "A few towns still persist in out-of-the-way places, but their numbers are limited." Davis (1960) reported on the prairie dog in Texas as, "Now rare throughout most of its former range." These undocumented descriptions do not adequately reflect the prairie dog population size in Texas.

The need for documented management data on this species and the capability to census colonies over vast areas provided the impetus for this study.

## SURVEY AREA

The survey area encompasses 108 counties in central and western Texas totaling 34,388,179 ha (343,615 $km^2$) (Soil Conservation Service, 1970) and covers the majority of the Kansan, Balconian, and Chihuahuan biotic provinces (Blair, 1950) (Fig. 1). Most of these counties are within the historical range of the prairie dog in Texas as determined by Bailey (1905) (Fig. 2).

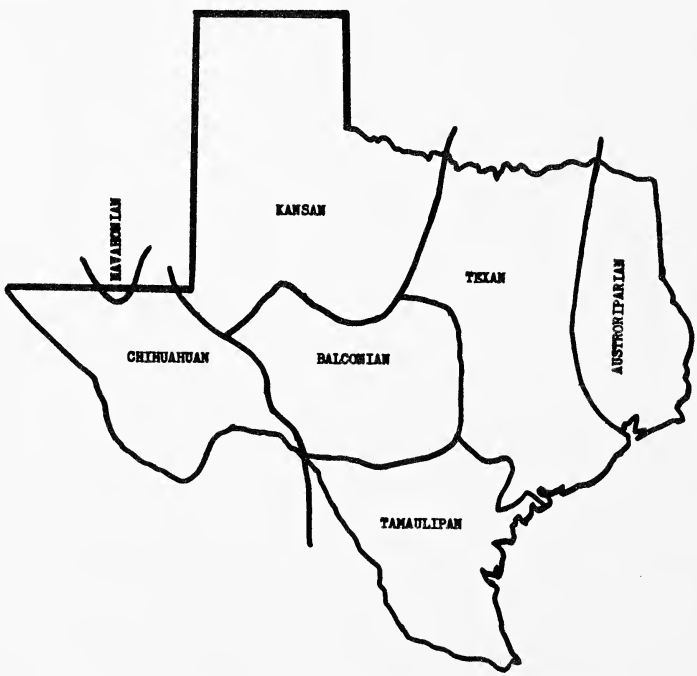


Figure 1. The biotic provinces of Texas as determined by Blair (1950).

Mesquite (*Prosopis juliflora*), juniper (*Juniperus* spp.), sand shinnery oak (*Quercus havardii*), sand sage (*Artemisia filifolia*), and scattered riparian vegetation occur throughout the area. Yucca (*Yucca galuca*), prickly pear (*Opuntia tuna*), and

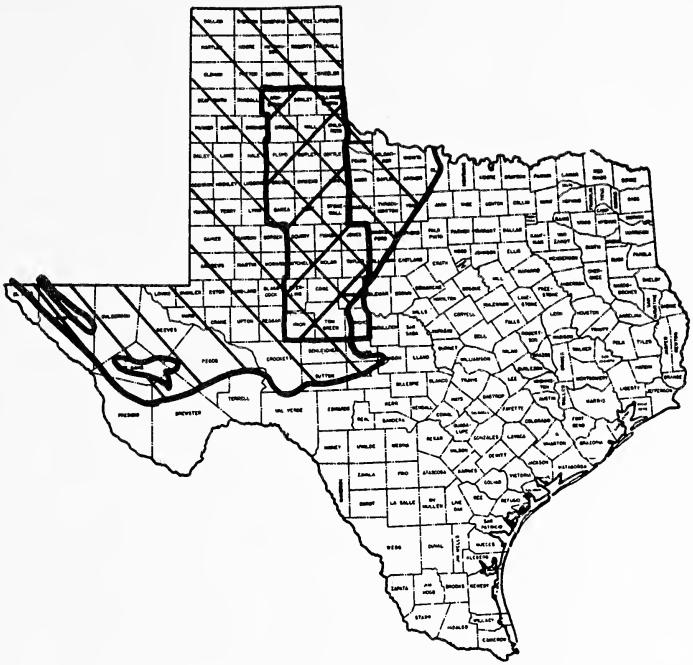

Figure 2. Prairie dog range in Texas and one continuous prairie dog colony described by Bailey (1905).

cholla (*Opuntia imbricata*) are common. The major grasses are 5 species of bluestem (*Andropogon* spp.), buffalo grass (*Buchloe dactyloides*), and 6 species of grama (*Bouteloua* spp.).

## METHODS

Agricultural Stabilization and Conservation Service (ASCS) aerial photography at a scale of 1:7,920 was used to locate colonies. Each photograph covers 10.36 $km^2$ (4 $mi^2$) and its accuracy is corrected to ±1%. Finding that black-tailed prairie dog colonies could be recognized on these photographs led to a survey technique for this species (Cheatheam, 1972).

Prairie dog colonies on the photographs appear as a grouping of small white dots; each dot represents a large mound or a large area denuded of vegetation around a prairie dog mound. Occasionally, short vegetation within the colony perimeter is reflected on the photographs as a white haze through the colony. Each colony's size was measured with a polar planimeter and the colony was then plotted on Texas Highway Department county maps with a notation of the colony's size.

An estimated 30,000 plus ASCS aerial photographs were examined in the study area for prairie dog colonies. Photographs were available for most of the

study area; however, complete county-wide coverage was not available in a few counties, because they had only small amounts of improved rangeland, and in the 9 counties west of the Pecos River. No problems were encountered in photographically censusing colonies in this xeric habitat as several colonies were recognized on the available photography and inspected on-site.

Because of incomplete photo coverage, this management survey was discussed with many reliable individuals including the: County Agricultural Extension Service, Texas Parks and Wildlife Department, Soil Conservation Service, ASCS, State and Federal Animal Damage Control personnel, and landowners. These individuals provided valuable information concerning many colonies that were not recognizable on photographs or that were in areas not photographed.

Of the total colonies located, 319 (23.9% of the total) were inspected on-site to verify the colony's presence, size, and location. The involved area was measured by automobile odometer or by pacing.

## RESULTS

The black-tailed prairie dog in Texas continues to occupy the same general area described by Bailey (1905); the present boundary was determined to be west and north of a line from Henrietta south through Palo Pinto, Coleman, and Cherokee and west through Sonora, Marathon, and Sierra Blanca (Fig. 3).

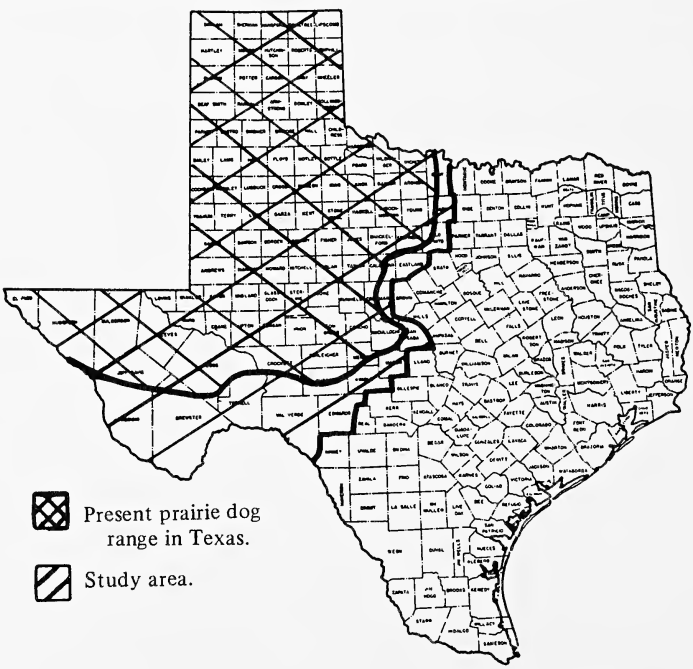


Figure 3. The present range parallels the species' 1905 range by Bailey (1905).

In the 108 county survey area, 1,336 prairie dog colonies were recorded totaling 36,432 ha in 89 counties (Fig. 3). Of this number, 1,144 colonies comprising 31,215 ha were located from aerial photographs. The remaining 192 colonies, consisting of 5,217 ha were located through contacts with individuals familiar with the area or while traveling to or from on-site inspections of other colonies. In counties with prairie dogs, the mean number of colonies was 15.16 ± 3.70, and the average area occupied by prairie dogs/county was 409.3 ha. The average colony occupied 27.27 ha. The smallest colony contained a few burrows. The largest was a Schleicher County colony that was estimated by the landowner to cover 7.8 $km^2$; accurate ground measurement of this colony was impossible due to its meandering boundary.

Counties in the Texas Panhandle bordering New Mexico have the greatest number of colonies. Dallam County (106 colonies, 3,514 ha) and Bailey County (80 colonies, 2,458 ha) have the largest populations in the State. Each county's population decreased progressively in an easterly direction to the fringe of the specie's range.

On-site inspections of 229 colonies located on the photographs indicated that: 45 colonies totaling 2,650 ha (8.5% of total) were no longer active; 13 colonies had decreased from 1,174 to 492 ha; and 30 colonies had increased from 1,068 to 2,899 ha. During on-site inspections, all the burrowing owls (*Speotyto cunicularia*) observed were tabulated because of their interest to scientists and their status as a colony-associated species.

Burrowing owls were not found on the colony surface in all seasons or weather extremes. This temporary absence may be further extended by their reproductive processes (Martin, 1973a), diurnal and crepuscular habits (Thomsen, 1971), migratory activities (Brenckle, 1936), or nocturnal activities; nevertheless, a total of 1,268 owls were counted in 91 colonies totaling 3,064 ha—an average of 1 owl/2.39 ha. That colonies containing owls were widely scattered throughout the study area indicates this average may be representative of the study area.

Time available for the study prevented lengthy investigations for the black-footed ferret (*Mustela nigripes*). During on-site colony inspections, no ferret or ferret signs, as described by Fortenberry (Biologist, U.S. Fish and Wildlife Service, pers. comm., 1971) and Hillman (1968), were observed.

## DISCUSSION

Many authors have not adequately described all components responsible for the reduction of the prairie dog from its historical population. It is a fact that land-use changes were, and continue to be, a factor in the decline.

The 89 counties in which prairie dog colonies were located comprise a total of 25,497,831 ha (254,978 $km^2$). Water areas (over 16.2 ha) and urban build-up totaled 657,177 ha or 2.6% of the land area. The remaining "Inventoried Area" (IA) (Fig. 4), 24,838,042 ha, is further divided as follows: cropland, 7,235,324 ha

(29.1% IA); rangeland, 16,539,115 ha (66.6% IA); and improved pasture, forest, and other, 1,063,603 ha (4.2% IA) (Soil Conservation Service, 1970). The rangeland figures for the 89 counties would not, in their entirety, be optimum prairie dog habitat due to land slope and soil composition. More importantly, the present land-use patterns outlined indicate a great loss of prairie dog habitat; nevertheless, on the basis of the rangeland figures, 1 ha of prairie dog colony exists per 182 ha of rangeland throughout the prairie dog range in Texas.

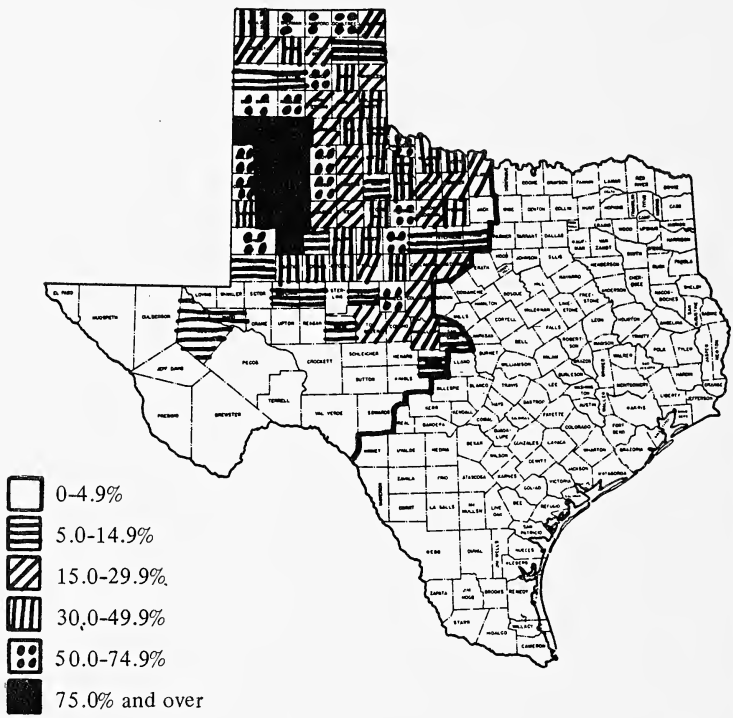


Figure 4. Percent of Inventoried Area (excludes water, urban build-up) of county in agricultural crops in study area (Soil Conservation Service, 1970).

Bailey (1905) described a single colony stating that, "They (prairie dogs) cover the whole country . . . from San Angelo (Tom Green County) north to Clarendon (Donley County) in a strip approximately 100 mi wide by 250 mi long (64,750 $km^2$)." This area was estimated and plotted on Fig. 3. The estimated area covers 65,783 $km^2$. Present urban build-up and water cover 1.9% of the area. The IA is: improved pasture, forest, and other uses, 2.6%; cropland, 27.5%; and rangeland, 69.9% (Soil Conservation Service, 1970). It is evident that this colony would be seriously disrupted under the present land-use patterns.

During the on-site inspections, it became apparent that colonies were continuing to be destroyed by changes in land use. The largest was a colony in Lynn County.

The 1969 aerial photos showed a 607 ha colony in a mesquite thicket. The 1972 site inspection showed an excellent stand of cotton in the former 7.8 $km^2$ pasture. Without nearby pastureland, this colony probably died from a lack of suitable habitat for dispersal or emigration.

However, the Kansan biotic province (Fig. 1) in Texas contains thousands of playa lakes in intensely farmed agricultural counties (Fig. 4). These unfarmed playas are inundated many times each year. Most of them contain some grass slopes between the lake bottom and plowed fields. In intensely farmed areas, such as Castro County, these playa edges constitute the majority of the available prairie dog habitat.

Castro County contains a total of 223,483 ha with only 28,382 ha of rangeland (Soil Conservation Service, 1970). Luther Geiger (Soil Scientist, Soil Conservation Service, pers. comm., 1973) reports that the county has over 500 playa lakes and that 6,459 ha are inundated many times each year. This leaves about 21,923 ha of rangeland for prairie dogs. The county has 41 colonies totaling 699 ha; most are small and occur in the fringe vegetation around playa lakes. Since prairie dogs in playa edge colonies do little harm, they are not controlled. The colony cannot expand due to the barriers of plowed fields and the lake bottom, and thus remain relatively static over the years. Extremely drastic land-use changes in playa lakes must occur to remove these reservoirs of prairie dog populations in this biota.

Several cities in the study area, such as Amarillo and Lubbock, and several cities outside the study area, such as Ft. Worth and Tyler, have small transplanted colonies in local parks that were not included in the survey. However, 6 cities in the study area, such as Plainview and Sweetwater, have colonies that appeared to naturally occur on vacant lots or grasslands within the cities' limits. A total of 10 colonies occupying 31 ha were located and included in this survey. Colonies in such locations have high esthetic value and their natural existence contributes to the State's native population. That such colonies occur may be viewed as an inherent tenacity of the species to continue perpetuation.

The State's native prairie dog population can only be estimated as the number of animals collectively inhabiting any colony are constantly fluctuating. Some relative influences affecting density/ha are: the abundance of lack of vegetation, weather, and counts made before or after parturition or winter mortality or the emigration of some adults. Merriam (1902) reported, "The number of holes on each acre varies from a few to upward of a hundred, and probably averages at least 25. It is certainly a conservative estimate to assume the average number of animals/acre to be 25." Assuming this estimate is presently valid, it may be stated that 2,249,000 prairie dogs inhabit the State and that they continue to occupy the same general area in Texas described at the turn of the century.

Such a population certainly cannot be classified as rare within its range in Texas or endangered as a species. That the prairie dog has continued is evident—that it will continue, is reasonable.

ACKNOWLEDGEMENTS

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# YIELD ESTIMATES DERIVED FROM ACTIVE AND PASSIVE CREEL SURVEYS OF A SMALL POND FISHERY[1]

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ABSTRACT

Estimates of angler harvest from a small pond were made by active and passive (voluntary-reporting) survey techniques. According to the active survey, anglers removed 789 fish weighing 61.3 kg (64.5 kg/ha) from the pond, made 577 fisherman visits to the pond, and caught 1.06 fish/hr during 1973-74. The passive survey method underestimated fishing pressure (no. anglers) and yield (no. fish) but gave an estimate of catch rate similar to that of the active surveys.

## INTRODUCTION

Fishery managers often rely upon either voluntary reporting of catch information or direct, in-the-field, creel surveys to estimate fish harvest rates. Such studies are usually confined to the largest and most important fisheries in the manager's region. Comparisons of harvest data collected by voluntary reporting, and an active creel survey in which the angler is actually observed by the biologist as a census technique, usually confirms the well-known tendency for anglers to exaggerate (Carlander, *et al.*, 1958; Carline, 1972; and Radford, 1973). During an intensive study of energy flow in a small pond ecosystem (Jones, 1975) we made estimates of fish harvest rates by both passive (voluntary reporting) and active creel survey methods and were subsequently able to compare the two sets of data in light of concurrent measurements of fish production and biomass in the pond.

## THE STUDY AREA

The study was conducted on a 0.95 ha pond located on the North Texas State University golf course. The pond is located just inside the city limits of

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Denton, Texas, off Interstate Highway 35 and on Avenue D. Avenue D, a gravel road, runs along the east side of the pond, 1.5-6.1 m from the shore. The other sides of the pond are bordered by the course fairways.

In most years the pond was capable of supplying all irrigation water needed by the golf course, but drawdowns in summer months sometimes removed 70% of the total storage volume. Prior to 1973, natural runoff to the pond was supplemented by water purchased from the City of Denton. In 1972, a well was drilled 30.6 m southeast of the pond to provide pumped water for the increased demands of the golf course. During this study, well water was pumped into the pond, but problems with the pumping mechanism caused drastic fluctuations in water level in summer and fall months. By late fall the pump was working properly and drawdowns were less severe.

The pond was constructed in 1947 and Bill White, fisheries manager at the Lewisville, Texas, fish hatchery, stocked bluegill sunfish (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and channel catfish (*Ictalurus punctatus*) in unknown numbers in 1948. Channel catfish were last taken from the pond in 1970, when two 4.5-kg specimens were captured, marked, and returned to the pond. No catfish were collected during this study. No additional fish have been stocked by the hatchery, but the superintendent of the golf course added 7 large (0.7-0.9 kg) white crappie (*Pomoxis annularis*) from Lake Dallas in 1970, and other additions are assumed to be accidental. During this study there were 7 species of fish in the pond: black bullhead (*Ictalurus melas*), largemouth bass, white crappie, bluegill, green sunfish (*Lepomis cyanellus*), longear sunfish (*Lepomis megalotis*), and golden shiner (*Notemigonus chrysoluecas*). Fishing in the pond was prohibited for many years until the summer of 1973, when fishermen were allowed to fish on the east side of the pond next to the road.

## METHODS AND MATERIALS

The passive creel survey began in July 1973 with the installation of two data boxes or registers on the east side of the pond in full view of anyone approaching from the road and concluded in May, 1974. The registers were equipped with cards for recording number of fishermen, gear used, number of hours fished, and number, species, and length of all fish caught. Pencils and rulers for measuring the fish were also provided. Lengths were converted to weights using length-weight relationships derived from seine samples. Signs posted near the boxes asked fishermen to fill in the cards and deposit them in the boxes with any fish tags collected. We tried to contact as many fishermen as possible and asked them to cooperate in the voluntary reporting.

The active creel survey employed a stratified monthly sampling regime similar to one described by Best and Boles (1956). At least 2 weekdays and 1 weekend day of each week were sampled in each month from June 1973 to May 1974. The selection of days within these restrictions was made randomly. Both mornings

and evenings were sampled on each day selected. Sampling was from 0600 hrs to 2100 hrs. At each 3-hr period we would travel to the pond and observe the fishermen for 1 hr. Data were collected on type of gear used, bait, number of fishermen, and amount of gear. The fishermen were checked for catch and those still fishing from the last period were questioned as to how long they had fished, and if any other fishermen had come and gone during the interval. Night fishing was prohibited by the golf course attendants and was not monitored beyond 2100 hrs. We believe there was very little illegal night fishing. Each category (weekday and weekend) value was then expanded to estimate total harvest figures for each month.

Fish collected for research purposes (stomach analysis, respiration studies, etc.) were tabulated and included in the total harvest figures.

## RESULTS

### *Active Creel Survey*

Although fishing was prohibited until June 1973, enforcement of the prohibition had always been rather lax and some fishermen seemed to make a practice of fishing for 30-60 min until asked to leave by the course attendants. When fishing was no longer prohibited after June 1973, these fishermen began to fish the pond steadily, and were joined by newcomers. Catches were high in June and July 1973, when 538 fish (68.2% of the annual catch of 789), amounting to 38.1 kg (62.2% of the total harvest weight of 61.3 kg) were caught (Table 1). The catch decreased steadily during the rest of the summer and fall. In winter months the catch rate increased slightly and then fluctuated somewhat during the late winter and spring.

Black bullhead made up 75.3% of the total catch by numbers (594 fish) and 83.2% (51.0 kg) of the catch by weight according to the active census. Adult bass(11) and crappie (19) were the largest fish captured, with a 2.36 kg crappie being the largest fish recorded. Although sunfish were the second most abundant fish captured, they accounted for only 3.3 kg of weight removed.

The number of fishermen using the pond decreased through the year. Of some 738 fishermen visits to the pond, 415 were in June and July 1973. The number of fishermen visits decreased during fall and winter, then increased again in spring, but never reached the high of the previous summer. Fishing occurred primarily in the afternoon from 1400 hr to sunset. Only 2 fishermen were observed fishing at night, and very little fishing occurred during the morning (6.5%). Weekend fishing was more popular than weekday fishing throughout the study. Summer fishermen used both cane poles and rod-and-reel combinations with natural or preserved food baits. During the fall and winter most fishermen used only 1 pole, while in summer and spring the average fisherman used 1.1-1.5 poles. One man tended 10 cane poles at one time. The highest catch rates (2.31 fish/hr) occurred in May. The average catch rate for the entire year was 1.06 fish/hr.

TABLE 1

Results of the Active Creel Survey; Data Presented as Number of Fish (Top) and Live Weight (kg, Bottom). All Fish Captured Were Removed from the Pond.

| | Hours Fished AM | Hours Fished PM | No. Fishermen | No. Gear | Black Bullhead | Bluegill Sunfish | Green Sunfish | Longear Sunfish | L.M. Bass | White Crappie | Total | Fish/hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jun | 19.1 | 357.6 | 208 | 317 | 333.0<br>25.3 | 38.0<br>0.7 | 82.0<br>1.4 | | 3.0<br>1.4 | 11.0<br>2.7 | 467.0<br>31.5 | 1.24 |
| Jul | 21.3 | 85.3 | 75 | 98 | 60.0<br>6.2 | | 8.0<br>0.1 | | 3.0<br>0.3 | | 71.0<br>6.6 | 0.67 |
| Aug | | 18.6 | 16 | 16 | 9.0<br>1.3 | | | | | | 9.0<br>1.3 | 0.48 |
| Sep | 8.1 | 31.2 | 23 | 23 | | | | | 5.0<br>2.3 | | 5.0<br>2.3 | 0.13 |
| Oct | | 9.9 | 8 | 8 | | | | | | | | 0.00 |
| Nov | | 24.0 | 48 | 48 | 8.0<br>1.3 | | | | | | 8.0<br>1.3 | 0.33 |
| Dec | | 11.6 | 8 | 8 | | | | | | | | 0.00 |
| Jan | | 26.8 | 20 | 20 | 28.0<br>4.2 | | 3.0<br>0.1 | | | | 31.0<br>4.3 | 1.16 |
| Feb | | 29.4 | 31 | 31 | 11.0<br>1.6 | | | | | | 11.0<br>1.6 | 0.37 |
| Mar | | 34.1 | 74 | 93 | 59.0<br>5.3 | | 3.0<br>0.1 | | | | 62.0<br>5.4 | 1.82 |
| Apr | | 12.0 | 22 | 24 | 6.0<br>0.6 | 2.0<br>0.1 | 2.0<br>0.1 | | | 8.0<br>0.3 | 18.0<br>1.1 | 1.50 |
| May | | 46.4 | 44 | 52 | 80.0<br>5.2 | 8.0<br>0.3 | 16.0<br>0.3 | 3.0<br>0.1 | | | 107.0<br>5.9 | 2.31 |
| Total | 48.5 | 696.9 | 577 | 738 | 594.0<br>51.0 | 48.0<br>1.1 | 114.0<br>2.1 | 3.0<br>0.1 | 11.0<br>4.0 | 19.0<br>3.0 | 789.0<br>61.3 | 1.06 |

*Passive Creel Survey*

Table 2 presents the data derived from the passive creel survey in which the fishermen voluntarily supplied information on catches. Only 112.8 hr of fishing and 138 fish, weighing 42.6 kg, were reported. Black bullhead accounted for

TABLE 2

Results of the Passive Creel Survey; Data Presented as Number of Fish (Top) and Live Weight (kg, Bottom). All Fish Were Presumably Removed from the Pond.

| | Hours Fished AM | Hours Fished PM | No. Fishermen | No. Gear | Black Bullhead | Sunfish | L.M. Bass | White Crappie | Total | Fish/hr |
|---|---|---|---|---|---|---|---|---|---|---|
| Jun | | | | | | | | | | |
| Jul | | | 1 | 1 | 1.0<br>1.4 | 12.0<br>3.0 | | | 1.0<br>1.4 | |
| Aug | 5.0 | 9.3 | 8 | 8 | 6.0<br>3.9 | | | | 18.0<br>6.9 | 1.26 |
| Sep | | 11.0 | 6 | 6 | 13.0<br>4.8 | | 5.0<br>6.4 | | 18.0<br>11.2 | 1.64 |
| Oct | | 7.0 | 2 | 2 | | | | | | |
| Nov | | 3.0 | 2 | 2 | 1.0<br>0.1 | | | | 1.0<br>0.1 | 0.33 |
| Dec | | 4.0 | 2 | 2 | | | 1.0<br>1.0 | | 1.0<br>1.0 | 0.25 |
| Jan | | 8.5 | 3 | 3 | | | | | | |
| Feb | | 7.5 | 4 | 4 | 4.0<br>3.7 | | | | 4.0<br>3.7 | 0.53 |
| Mar | | 15.5 | 5 | 5 | 10.0<br>1.8 | | | | 10.0<br>1.8 | 0.65 |
| Apr | 6.0 | 26.0 | 13 | 13 | 17.0<br>3.9 | 27.0<br>2.4 | 2.0<br>0.5 | | 65.0<br>9.7 | 2.03 |
| May | 1.0 | 9.0 | 5 | 5 | 20.0<br>6.8 | | | 19.0<br>2.9 | 20.0<br>6.8 | 2.00 |
| Total | 12.0 | 100.8 | 51 | 51 | 72.0<br>26.4 | 39.0<br>5.4 | 8.0<br>7.9 | 19.0<br>2.9 | 138.0<br>42.6 | 1.22 |

most of the catch (72 fish; 26.4 kg), followed by sunfish (39 fish; 5.4 kg), largemouth bass (8 fish; 7.9 kg) and crappie (19 fish; 2.9 kg). No fish were reported in October and January, while only 1 fish/month were reported in July, November, and December. The low value in July is attributed to the fact that many fishermen were still unaware of the survey.

The late summer catch rates reported in the passive survey (1.26-1.64 fish/hr) were comparable to those determined in the active survey. During the fall and winter catch rates declined to 0-0.33 fish/hr and then increased steadily to 2.03 fish/hr in April. The mean catch rate over the entire year was 1.22 fish/hr.

*Fish Removed for Research*

Many fish were removed from the pond for research purposes (i.e., stomach analysis and respiration studies conducted by other students). Table 3 presents a summary by species of the 5,662 fish removed for research purposes (28.9 kg), which was almost half as much as that cropped by fishermen (61.3 kg).

TABLE 3
Number and Weight of Fish Removed from the Pond for Experimental Purposes.

| Species | Number | Weight (kg) |
|---|---|---|
| Black Bullhead | 2,992 | 5.9 |
| Bluegill | 554 | 4.0 |
| Green Sunfish | 824 | 7.7 |
| Longear Sunfish | 39 | 0.5 |
| Largemouth Bass | 246 | 2.3 |
| White Crappie | 842 | 5.9 |
| Golden Shiner | 165 | 2.6 |
| Total | 5,662 | 28.9 |

Of the fish removed for research purposes, green sunfish accounted for the largest weight removed (7.7 kg), followed by bullhead and crappie (5.9 kg each). The total removals of bluegill, golden shiner, largemouth bass, and longear sunfish were much lower (0.5-4.0 kg).

## DISCUSSION

The pond illustrated a classic example of a new fishery, with very high initial fishing and catch rates, followed by sharp reductions as fishing increased above optimum for the system. From catch data it was obvious that many of the large bullheads were fished out by late August, as were the bass and crappie.

The active creel survey has usually been considered to be more reliable than the passive or voluntary-reporting survey (Best and Boles, 1956; Carlander, *et al.*, 1958; Stewart and Trout, 1973). In this study the active survey resulted in an estimated harvest of 789 fish weighing 61.3 kg, while the passive survey reported only 138 fish weighing 42.6 kg. Thus the passive survey reported only 17% of the number of fish estimated by the active survey, but the weight reported in the passive survey was nearly 70% of that obtained in the active survey. These discrepancies probably result from the tendency of fishermen to report only catches of large fish, to overestimate the size of fish taken, or both. The passive

survey also underestimated the total amount of fishing pressure on the pond (51 fisherman visits compared to 577 for the active survey). Catch rate ranges reported by the 2 methods were comparable (0-2.31 fish/hr for the active and 0-2.03 for the passive).

The active survey estimate of total annual harvest rate was 830 fish/ha or 64.5 kg/ha. These figures are several times greater than the 62.8 fish/ha and 16.4 kg/ha mean annual sport fishing harvest rates reported for 130 U.S. reservoirs by Jenkins and Morais (1971). The reservoirs surveyed by Jenkins and Morais were all much larger (<202 ha) than the golf course pond and might be expected to yield fewer fish/ha. Bennett (1971) and Childers and Bennett (1967) have reported annual angler harvest rates of 3-76.7 kg/ha for fishes in ponds of 1-10 ha in the midwestern and southwestern U.S., although yields exceeding 50 kg/ha were rare. The annual harvest from the golf course pond in 1973-74 ranks among the highest for an uncultured system in the United States.

Jones (1975) reported a total annual production for all species of fish in the pond of 1,986 kg or 2,090 kg/ha, and a mean standing crop of 1,050 kg or 1,105 kg/ha. Therefore, fishermen removed only 3.1% of the total annual production. If we include the removals for experimental purposes in the annual harvest, it still amounts to only 4.5% of the annual production. However, Jones (1975) has also pointed out that most (83.8%) of the total annual production in the pond was laid on by young-of-the-year fishes which were not susceptible to the fishermen's gear. The annual yield to fishermen was 28.5% of the annual production of adult fish alone (Age Class I+).

We suggest that the passive creel survey method will greatly underestimate fishing effort and total harvest in a sport fishery, although it may give an accurate estimate of the catch rate. Yield to anglers in the pond was very high in the first year of legalized fishing, but probably cannot be expected to be maintained in subsequent years.

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# AGE AND GROWTH OF LARGEMOUTH BASS IN CANYON RESERVOIR, TEXAS

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ABSTRACT

The method of aging largemouth bass by their scales was validated for a central Texas reservoir. From March 1974 to April 1975, 838 fish were collected from Canyon Reservoir with bass tournaments being the major source of fish 305 mm or longer in total length. No substantial difference was found in the growth rates of males and females, however, the oldest females found were 6 years old and the oldest male was 5 years old, with age groups 4 and 5 containing mostly females. Mean total lengths (mm) of combined sexes for age groups 1-6 were 161, 269, 326, 392, 459, and 510, respectively. Mean weight increments (g) of combined sexes for age groups 1-6 were 45, 175, 179, 308, 446 and 446, respectively. Growth rates of largemouth bass from Canyon Reservoir are compared to those from other states. The length-weight relationship was log $W$ = -5.2005 + 3.1039 log $L$, where $W$ = weight in g and $L$ = total length in mm, and the bass exhibited allometric growth. A condition factor of 4.0 (SE = 0.02) indicated the fish were in poor flesh.

## INTRODUCTION

Age and growth studies have been used as fisheries management tools for many years and in many parts of the U.S. They have been used not only to determine the existence of a problem, but also to evaluate the results of applied management practices.

The scale method of determining the age and growth of fishes is generally accepted as valid for fishes in northern states. However, its validity for fishes in southern states has been in question for many years due to mild winters (Prather, 1967), since for an annulus to be formed, growth during the winter must cease for at least 14 days (Bennett, 1970).

The objectives of this study of largemouth bass, *Micropterus salmoides,* in Canyon Reservoir were to validate the use of the scale method for aging largemouth bass in Canyon Reservoir; to determine the age composition, length-weight relationship, and condition factor.

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## METHODS AND MATERIALS

Canyon Reservoir is a deep-storage impoundment in Comal County about 26 km west of San Marcos, Texas. It was impounded in June 1964 and has a 3,300 ha surface area and 40 m maximum depth.

Specimens were collected from March 1974 to April 1975 by seining, hook and line, rotenoning of coves and gill netting. Bass tournaments were the major source of fish 305 mm (12 in) or longer; most smaller fish were taken by seining. Letters sent to bass clubs within a 210-km radius of the study area requested permission to obtain data from fish caught in their Canyon Reservoir tournaments. Many clubs responded to this request and were very cooperative at the tournament weigh-ins.

When possible, total length (mm), weight (g) and sex were determined for each specimen, recorded on a numbered scale envelope and a scale sample from that individual placed in the envelope. The scale sample contained 5 to 8 scales removed from a key area located at the tip of the depressed left pectoral fin and 2 to 4 scale rows below the lateral line. Scale impressions were made on a cellulose acetate slide with a roller press (Smith, 1954) and the slide identified by number. The impressions were examined using a scale reading machine (Van Oosten, *et al.*, 1934), without knowledge of the size of the fish from which the scales came. All slides were reread without knowledge of the first or subsequent readings until there was agreement between two readings for each slide. The slides were examined in groups of at least 75 to reduce the possibility of bias due to remembering previous examinations of the same slide. According to Carlander (1961), repetition of readings may increase the accuracy of age determinations.

After the age of fish was determined, the positions of the focus, each annulus and the margin of a representative scale measured along the midanterior field of the scale at 60x were marked on a strip of paper along with the fish identification number, total length and sex. Each strip of paper was used in a nomograph method for the back-calculation of the total length of the fish at the end of each year of life (Carlander and Smith, 1944). To determine the $y$-intercept on the nomograph, a body-scale relationship was calculated on measurements from 686 largemouth bass from Canyon Reservoir using the following equation:

$$L = a + bS$$

where $L$ is the total length (mm) of the fish, $S$ is the distance from the focus to the anterior margin of the scale (mm x 60), $a$ is the $y$-intercept on the nomograph and $b$ is the slope. Since scale measurements were used to derive total lengths of fish, the scale measurements were designated the independent variable (Whitney and Carlander, 1956). Characteristics used for the recognition of annuli and false annuli were according to Prentice and Whiteside (1975).

A length-weight relationship was calculated on data from 838 largemouth bass of both sexes with total lengths which ranged from 34 to 563 mm with a

mean of 179 mm and weights which ranged from 0.6 to 2523 g with a mean of 64 g by using the formula:

$$\log W = a + b \log L$$

where $W$ is the weight (g), $L$ is the total length (mm), $a$ is the $y$-intercept and $b$ is the slope. A two-tailed student's $t$-test was used to test the hypothesis that the slope of the length-weight relationship line was equal to 3.0 (Steel and Torrie, 1960).

A condition factor for all fish was calculated according to the following equation:

$$\text{C.F.} = \frac{10{,}000\ W}{L^3}$$

where $W$ is the weight (g) and $L$ is the total length (mm) (Bennett, 1970).

## RESULTS AND DISCUSSION

*Length-Weight Relationship and Condition Factor*

If the slope of a length-weight relationship line is close to 3.0, fish growth is isometric (no change in body shape with a change in length), and the condition factor should not vary with changes in length of the fish. If the slope is not equal to 3.0 then growth is allometric (a change in body shape with a change in length), and the condition factor should vary with changes in length of the fish. However, in both types of growth there is individual variability (Tesch, 1968; Rounsefell and Everhardt, 1962). The length-weight relationship equation for largemouth bass from Canyon Reservoir was:

$$\log W = -5.2005 + 3.1039 \log L.$$

The difference between the slope of the length-weight relationship line for largemouth bass in Canyon Reservoir ($b = 3.1039$) and 3.0 was highly significant ($p < 0.001$; 836 df), indicating that there should have been an increase in the condition factor with an increase in length. An increasing condition factor trend with an increase in length was also found, further indicating that growth was allometric.

Bennett (1970) gave the following ranges of condition factors for largemouth bass between 127 and 381 mm in total length: 3.5-4.5 = a fish in poor flesh, 4.6-5.5 = a fish in average flesh, 5.6-6.5 = a very fat fish. The mean condition factor for 462 largemouth bass between 127 and 381 mm in total length in Canyon Reservoir was 4.2 (SE = 0.03). According to Bennett's ranges, the bass population in Canyon Reservoir was below average condition, indicating a potential growth problem. The overall mean condition factor was found to be 4.0 (SE = 0.02) for 804 largemouth bass from Canyon Reservoir between 34 and 563 mm in total length.

A total length (TL) to standard length (SL) conversion equation, SL = 1.7379 + 0.8195 TL, was calculated from 128 Canyon Reservoir largemouth bass ranging in total length from 60 to 522 mm.

*Aging Trials*

Although criteria from recognizing annuli and supernumerary marks are well established, some scale marks were difficult to interpret. Age analysis results (Table 1) show that, although agreement of two readings was obtained by the second reading for a majority of fish (70%), many fish required a third reading (26%) and a few fish required a fourth reading (4%). The necessity for more readings with an increase in age was due to increased difficulty in distinguishing annuli that formed closer together in later years of life. This same phenomenon was found by Prentice and Whiteside (1975) in a study of largemouth bass of known age in central Texas farm ponds, yet they were able to attain a 94% aging accuracy.

TABLE 1

Number and % Agreement of Aging Trials for Age Groups of Largemouth Bass Collected from Canyon Reservoir, Texas, in 1974-75.

| Age | No. of fish in age group | No. of individuals with agreement of 2 readings by | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2nd Reading | | 3rd Reading | | 4th Reading | |
| 1 | 111 | 79 | (71)[a] | 29 | (97)[a] | 3 | (100)[a] |
| 2 | 312 | 244 | (78) | 61 | (98) | 7 | (100) |
| 3 | 115 | 69 | (60) | 39 | (94) | 7 | (100) |
| 4 | 42 | 19 | (45) | 19 | (90) | 4 | (100) |
| 5 | 9 | 2 | (22) | 4 | (67) | 3 | (100) |
| 6 | 5 | 0 | ( 0) | 3 | (60) | 2 | (100) |
| Total | 594 | 413 | (70) | 155 | (96) | 26 | (100) |

[a]Numbers in parentheses are % agreement by that reading.

*Validation of Scale Method*

Many fisheries workers assume that Texas fishes do not form annuli each year, therefore, the scale method has seldom been used in the area. Silvey and Harris (1948) found that the scale method of age determination for largemouth bass was reliable in east Texas provided that a sufficient number of fish were examined. Prentice and Whiteside (1975) studied the validity of aging largemouth bass in central Texas farm ponds and showed that the criteria for validation of the scale method were satisfied.

To validly use the scale method for age determination it is necessary to determine the approximate time and regularity of annulus formation each year (Hile, 1941; Berg and Grinnaldi, 1967). A small percentage of Canyon Reservoir largemouth bass appeared to have formed annuli by the latter part of January and February, a large percentage formed annuli during March and most had formed annuli by the end of April or May (Table 2). A trend in the time of annulus formation for different age and length groups could not be determined due to small numbers of fish collected each month.

To check for regularity of annulus formation, back-calculated total lengths of bass at different ages were examined for similarity to the empirical total lengths

TABLE 2

Time of Annulus Formation for Largemouth Bass Collected from Canyon Reservoir, Texas, in 1974-75.

| Collection Date | Total No. Collected | % Fish With Annulus |
|---|---|---|
| March 29 & 30, 1974 | 19 | 47 |
| May 5, 1974 | 150 | 100 |
| May 17, 1974 | 14 | 100 |
| January 22 & 24, 1975 | 18 | 11 |
| February 21, 1975 | 27 | 11 |
| March 19, 1975 | 18 | 33 |
| March 29, 1975 | 8 | 38 |
| April 6 & 9, 1975 | 56 | 59 |
| April 12, 1975 | 53 | 70 |
| April 27, 1975 | 34 | 94 |

of bass at that age. Back-calculated total lengths of fish were obtained by using a nomograph method with a $y$-intercept correction of 36.7 mm ($L = 36.715 + 1.087\ S$). The mean empirical total lengths (mm) of the bass in age groups 1+ through 6+ were found to be 245, 303, 351, 409, 469 and 517 respectively, and were similar to the back-calculated grand mean total lengths (weighted for individuals) of the bass at each corresponding annulus (Table 3).

*Comparison of Age and Growth Between Sexes*

No substantial difference in growth rates between male and female bass was found when using a common $y$-intercept correction factor for back-calculating total lengths (Table 4). Separate $y$-intercept correction factors for back-calculation of total lengths for each sex were not calculated since few small bass could be sexed. Stroud (1948), in a study of Norris Reservoir, Tennessee, found that older and larger largemouth bass tended to be females. Padfield (1951), in a study in Alabama, found that male largemouth bass were few or absent in the older age groups. In Canyon Reservoir the relative abundance of males showed a sharp decrease in age group 4 and males were absent in age group 6 (Table 4).

*Comparison of Growth Rates in Canyon Reservoir with Those of Other Areas in the United States*

In many age and growth studies of largemouth bass, a zero $y$-intercept was assumed when back-calculating lengths of fish. For the purpose of comparison with these studies and studies that used a corrected $y$-intercept, total lengths were back-calculated using both a zero $y$-intercept and a corrected $y$-intercept (Table 3). Largemouth bass in age groups 1 through 4 from Canyon Reservoir had lower growth rates than largemouth bass from Louisiana, Oklahoma, Arkansas and Tennessee, while older age groups had growth rates similar to those from Oklahoma, Arkansas and Tennessee (Table 5). However, the growth rates of largemouth bass in Canyon Reservoir were greater than those in more northern states.

TABLE 3

Back-calculated total lengths (mm) at the end of each year of life for largemouth bass collected from Canyon Reservoir, Texas, in 1974-75. Total lengths were obtained using a corrected $y$-intercept of 36.7 mm. Numbers of individuals used in calculations are given in parentheses.

| Year Class | Age Groups 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1974 | 181 (16) | | | | | |
| 1973 | 177 (248)' | 283 (153) | | | | |
| 1972 | 155 (191) | 281 (191) | 352 (32) | | | |
| 1971 | 137 (94) | 237 (94) | 325 (94) | 448 (11) | | |
| 1970 | 135 (35) | 229 (35) | 310 (35) | 373 (35) | 478 (4) | |
| 1969 | 162 (5) | 247 (5) | 304 (5) | 390 (5) | 440 (5) | |
| 1968 | 140 (5) | 251 (5) | 340 (5) | 404 (5) | 462 (5) | 510 (5) |
| Grand mean total length (weighted for individuals) | 161 | 269 | 326 | 392 | 459 | 510 |
| Grand mean total length increment | 161 | 108 | 57 | 66 | 67 | 51 |
| [a]Grand mean weight increment | 45 | 175 | 179 | 308 | 446 | 446 |
| Mean annual total length increment (weighted for year classes) | 161 | 104 | 77 | 84 | 71 | 48 |
| Sum of mean annual total length increment | 161 | 265 | 342 | 426 | 497 | 545 |
| [b]Grand mean total length (weighted for individuals) | 142 | 260 | 324 | 389 | 457 | 509 |
| Total number of fish | 594 | 483 | 171 | 56 | 14 | 5 |

[a]These weight increments were calculated using the length-weight relationship equation.
[b]These back-calculated total lengths were obtained using an assumed zero $y$-intercept.

The general increase in the rate of growth of fish from north to south is usually attributed to an increase in the length of growing season (Bennett, 1937; Viosca, 1942; Stroud, 1948; Jenkins and Hall, 1953). However, Jenkins (1974), in an analysis of large reservoirs in the southern U.S., made some general statements concerning the factors governing the productivity of water. Generally the highest

TABLE 4

Back-calculated total lengths (mm) at the end of each year of life for male and female largemouth bass collected from Canyon Reservoir, Texas, in 1974-75. Total lengths were obtained using a corrected $y$-intercept of 36.7 mm. Number of individuals used in calculations are given in parentheses. M = male, F = female

| Year Class | Age Groups 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| 1974 | 195 (9) | 191 (5) | | | | | | | | | | |
| 1973 | 182 (126) | 187 (78) | 283 (105) | 282 (48) | | | | | | | | |
| 1972 | 156 (90) | 156 (96) | 280 (90) | 276 (96) | 345 (21) | 364 (11) | | | | | | |
| 1971 | 134 (48) | 139 (46) | 231 (48) | 243 (46) | 317 (48) | 333 (46) | 437 (3) | 452 (8) | | | | |
| 1970 | 141 (10) | 133 (25) | 233 (10) | 227 (25) | 319 (10) | 306 (25) | 373 (10) | 373 (25) | 458 (1) | 484 (3) | | |
| 1969 | -- | 162 (5) | -- | 247 (5) | -- | 304 (5) | -- | 390 (5) | -- | 440 (5) | | |
| 1968 | -- | 140 (5) | -- | 251 (5) | -- | 340 (5) | -- | 404 (5) | -- | 462 (5) | -- | 510 (5) |
| Grand mean total length (weighted for individuals) | 164 | 161 | 270 | 264 | 325 | 328 | 388 | 393 | 458 | 459 | -- | 510 |
| Grand mean total length increment | 164 | 161 | 106 | 103 | 55 | 64 | 63 | 65 | 70 | 66 | -- | 51 |
| Mean annual total length increment (weighted for year classes) | 164 | 161 | 104 | 102 | 79 | 81 | 87 | 84 | 85 | 73 | -- | 48 |
| Sum of mean annual total length increment | 164 | 161 | 268 | 263 | 347 | 344 | 434 | 428 | 519 | 501 | -- | 549 |
| Total number of fish | 283 | 260 | 253 | 225 | 79 | 92 | 13 | 43 | 1 | 13 | -- | 5 |

production of sport fishes occurs where all of the following criteria are found: (1) mean annual concentration of total dissolved solids is from 120-350 ppm, (2) dominant ions are calcium-magnesium and carbonate-bicarbonate and (3) mean annual suspended sediment load of inflowing streams is less than 2000 ppm. Hannan and Young (1974), Jenkins (1974) and Rawson (1968) indicated that the Canyon Reservoir environment met the three above criteria. Jenkins (1974), further stated that if the above criteria are met then the length of the growing season is the major factor controlling sport fish production and Canyon Reservoir has a growing season of approximately 260 days.

TABLE 5

Comparison of grand mean total lengths weighted for individuals (mm) of largemouth bass in U.S. waters. An assumed zero $y$-intercept was used in calculating lengths.

| Location | Reference | Age Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Central Texas | This study | 142 | 260 | 324 | 389 | 457 | 509 | | |
| Louisiana | Carlander, 1950 | 193 | 287 | 368 | 478 | 531 | 597 | 630 | 658 |
| Oklahoma (Reservoir mean) | Jenkins and Hall, 1953 | 170 | 290 | 361 | 417 | 465 | 498 | 528 | 554 |
| Bull Shoals Reservoir, Ark. | Bryant and Houser, 1971 | 176 | 297 | 377 | 427 | 457 | 492 | 519 | 524 |
| Norris Reservoir, Tenn. | Stroud, 1948 | 175 | 310 | 371 | 406 | 445 | 490 | 528 | |
| Nebraska | Carlander, 1950 | 91 | 193 | 277 | 343 | 401 | 447 | 480 | 503 |
| Ohio | Carlander, 1950 | 89 | 178 | 257 | 318 | 368 | 409 | 450 | 480 |
| Wisconsin | Bennett, 1937 | 94 | 188 | 267 | 318 | 356 | 384 | 414 | 442 |
| Minnesota | Carlander, 1950 | 88 | 170 | 236 | 292 | 333 | 384 | 414 | 447 |

It should be pointed out that just because the younger age groups of largemouth bass in Canyon Reservoir have slower growth than other southern reservoirs, that this does not mean that the bass production in Canyon Reservoir is low. In fact, based upon Jenkins' (1974) criteria a high production of bass would be expected. Thus, it should be kept in mind that production is dependent upon many factors (i.e., growth rates, mortality rate, population size, etc.).

During the years immediately after the impoundment of Canyon Reservoir, catches of "large" largemouth bass were commonly reported by fishermen. However, during the present study many fishermen reported catching large numbers of bass but few large enough to weigh-in at bass tournaments. Gill-net data from Canyon Reservoir (J. Dean, Texas Parks and Wildlife Department, personal communication) showed a decline in the mean weight of largemouth bass from 748 g in 1969 to 227 g in 1975. Bennett (1970) stated that "in the production of fishes above average size, a large source of available food per individual fish seems to exceed in importance the length of the growing season." Many fisheries managers assume that in southern waters available food is usually not limiting, which may not be true, since a problem in many southern reservoirs is the overpopulation of game and rough fish (Thompson, 1954) causing high intraspecific and interspecific competition.

It is our understanding that in referring to the forage fishes available to the sport fishes, that many biologists include adult forage fishes of which many are too large to be utilized by most sport fishes. Thus, a large portion of the rough fish population is not available as a food source but is actually competing with sport fishes for certain types of food. The small average size of bass in Canyon

Reservoir may be due to a large population of bass and a limited supply of food for which the bass must compete.

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# THE EFFECTS OF AMNIOTIC FLUID ON THE GROWTH OF CERTAIN GRAM-NEGATIVE BACTERIA

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ABSTRACT

The ability of bovine amniotic fluid to support the growth of 4 species of gram-negative bacteria was examined and compared with the ability of control laboratory medium to do so. The results indicate that growth is minimal in amniotic fluid and that this restriction is due to the presence of unidentified inhibitory substance(s) in the amniotic fluid rather than to the absence of essential nutrients in it. Furthermore, the antibacterial substance(s) remain stable when stored over long periods of time and when exposed to extreme temperatures.

## INTRODUCTION

Amniotic fluid, the watery medium which surrounds the embryo and fetus, has been shown to be of significant value to the developing unborn (Fairweather and Eskes, 1973; Moore, 1973; Natelson, *et al.*, 1974). Its protective role for the embryo or fetus is well documented (Balinsky, 1975). It is a dynamic fluid with large volumes moving in both directions between the fetal and maternal circulations, mainly via the placental membrane (Barnes and Seeds, 1972).

Amniotic fluid samples may be collected relatively easily from the amniotic cavity by transabdominal amniocentesis. This common clinical procedure has not been accompanied by an increased incidence of amnionitis (Galask and Snyder, 1968). Thus, the antibacterial properties of amniotic fluid have become the subject of many scientific investigations.

Walsh, *et al.*, (1965) and Sarkany and Gaylarde (1968) have suggested that amniotic fluid will support bacterial growth as well as will control laboratory medium. Kitzmiller, *et al.*, (1973) have claimed that amniotic fluid, in itself, is nutritionally deficient and will not support bacterial growth unless it is enriched with added nutrients. Galask, *et al.*, (1974) and Bergman, *et al.*, (1972) maintain that amniotic fluid is nutritionally sufficient for bacterial growth, but will not support bacterial growth due to the presence of antibacterial factors found in the fluid. These include such potential antibacterial substances as lysozyme, transferrin, immunoglobulins, and the beta-lysin, and the hormone progesterone.

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In view of the many conflicting reports in the literature concerning the antibacterial activity of amniotic fluid, this investigation was undertaken to determine the nature of the inhibitory effects of bovine amniotic fluid on the growth of four species of gram-negative bacteria.

## MATERIALS AND METHODS

### *Amniotic Fluid*

Amniotic fluid was collected aseptically from pregnant cattle. The fluid was collected into sterile plastic containers, and transferred into sterile Erlenmeyer flasks and immediately refrigerated at 5 C.

A 1-ml aliquot from each amniotic fluid sample collected was transferred into each of 4 culture tubes containing nutrient media to confirm the sterility of the amniotic fluid samples.

### *Organisms*

The test organisms, *Pseudomonas fluorescens, Proteus vulgaris, Escherichia coli,* and *Serratia marcescens,* were obtained from the microbiology stock culture collection of Southwest Texas State University. These organisms were subcultured and maintained on tryptic soy agar slants. From these slants, the organisms were cultured in tryptic soy broth.

### *Media*

Dehydrated Difco-Bacto tryptic soy broth and Difco-Bacto thioglycollate medium, without indicator, were rehydrated according to the manufacturer's instructions.

Two % (w/v) Difco-Bacto agar was added to the tryptic soy broth for making pour plates. Following sterilization, the media was placed in a 43-45 C water bath until used (within 2-3 hrs). Pour plates were incubated 24 hrs at 37 C.

Physiological saline (0.85% NaCl) and phosphate buffer (0.1 M; pH 8.0), used to dilute amniotic fluid and other media, were sterilized by filtration through 0.45 micron millipore filters. These sterile solutions were refrigerated at 5 C and were used within 1 week.

### *Experimental Procedures*

To determine the inhibitory capacity of amniotic fluid, growth studies were conducted on 4 species of bacteria by both direct (standard plate count) and indirect (photometric) methods of measurement.

*Dilutions of amniotic fluid.* Dilutions of amniotic fluid were made to determine whether the inhibitory properties of amniotic fluid could be diluted out before the loss of nutritional adequacy. Serial dilutions were made in 1-ml increments from 0 to 10 ml of amniotic fluid/culture tube. Phosphate buffer or physiological saline was pipetted aseptically into each of the culture tubes in the series to a final volume of 10 ml/tube.

*Enrichment of amniotic fluid.* Dilutions of amniotic fluid were made and enriched with tryptic soy broth to determine whether the amniotic fluid was nutritionally deficient. Serial dilutions were made in 1-ml increments form 0 to 10 ml of amniotic fluid/tube. Tryptic soy broth was pipetted aseptically into each of the series of culture tubes to a final volume of 10 ml.

*Effect of the inocula.* To minimize the lag phase, some species of bacteria used for inoculation were cultured in amniotic fluid instead of tryptic soy broth. These organisms were inoculated into tubes containing amniotic fluid or dilutions thereof. The *E. coli, Ps. fluorescens,* and *P. vulgaris* used in these experiments were cultured in 100%, 50% and 10% solutions of amniotic fluid. Sterile physiological saline was used to dilute all tubes to a final volume of 10 ml.

*Effect of prolonged incubation time.* Dilutions of amniotic fluid were made using sterile physiological saline and these dilutions were inoculated and cultured in the same manner as described previously, with the exception that the incubation period was extended over a time period of 72 hrs. Growth of the bacteria was measured photometrically and by the standard plate count method.

*Heat resistance of amniotic fluid.* The inhibitory activity of amniotic fluid was measured after the fluid was heated either to 100 C for 45 min or to 121 C for 15 min. These results were compared with the amniotic fluid samples that had been refrigerated at either 5 C or -20 C. *E. coli* was grown in 100%, 50%, and 10% solutions of each sample of amniotic fluid. Sterile physiological saline was used as the diluent in order to obtain a final volume of 10 ml/tube. Each tube was inoculated with 0.3 ml of *E. coli.* Bacterial growth was determined photometrically.

*Effect of fetal gestation age on amniotic fluid.* An attempt was made to correlate the inhibitory activity of amniotic fluid with the gestation age. Comparisons were made of the antibacterial activities of 3 different aged samples of amniotic fluid. All tubes were inoculated with 0.3 ml of *E. coli* from a young broth culture. Bacterial growth was determined photometrically.

*Long-Term stability of the amniotic fluid inhibitor(s).* Several samples of amniotic fluid were stored at 5 C for a period of 15 weeks to determine the stability of the bacterial inhibitory factor(s). Prior to storage, growth curves of *P. vulgaris* were made by inoculating the amniotic fluid with 0.3 ml of a young broth culture.

## RESULTS

### *Dilutions of Amniotic Fluid*

Amniotic fluid dilutions were made and were inoculated with the 4 species of bacteria used in this study. The results of these experiments are shown in Table 1. *E. coli* showed maximum log growth in the 50-60% dilutions of amniotic fluid while *P. vulgaris* and *S. marcesens* demonstrated maximum log growth in the 80-90% dilutions. *Ps. fluorescens* grew maximally in the 40-50% dilutions of amniotic

fluid. *E. coli* diluted with physiological saline demonstrated maximum growth in the 80% dilution of amniotic fluid.

TABLE 1

The effect of the dilution of amniotic fluid with phosphate buffer (PB) and physiological saline (S) on bacterial growth.

| | NET KLETT UNIT CHANGE PER 24 HRS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | % of Amniotic Fluid | | | | | | | | | | |
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
| *E. coli* (PB) | 5 | 10 | 21 | 32 | 48 | 67 | 71 | 65 | 62 | 67 | 60 |
| *Ps. fluorescens* (PB) | 9 | 27 | 33 | 42 | 54 | 47 | 38 | 38 | 30 | 32 | 37 |
| *P. vulgaris* (PB) | 14 | 11 | 11 | 14 | 12 | 20 | 29 | 33 | 39 | 35 | 10 |
| *S. marcescens* (PB) | 5 | 16 | 25 | 35 | 38 | 51 | 57 | 58 | 61 | 63 | 56 |
| *E. coli* (S) | 4 | 17 | 30 | 37 | 53 | 54 | 50 | 50 | 75 | 45 | 50 |

Growth rates of the organisms cultured in 100% amniotic fluid were compared to the tryptic soy broth controls (Fig. 1).

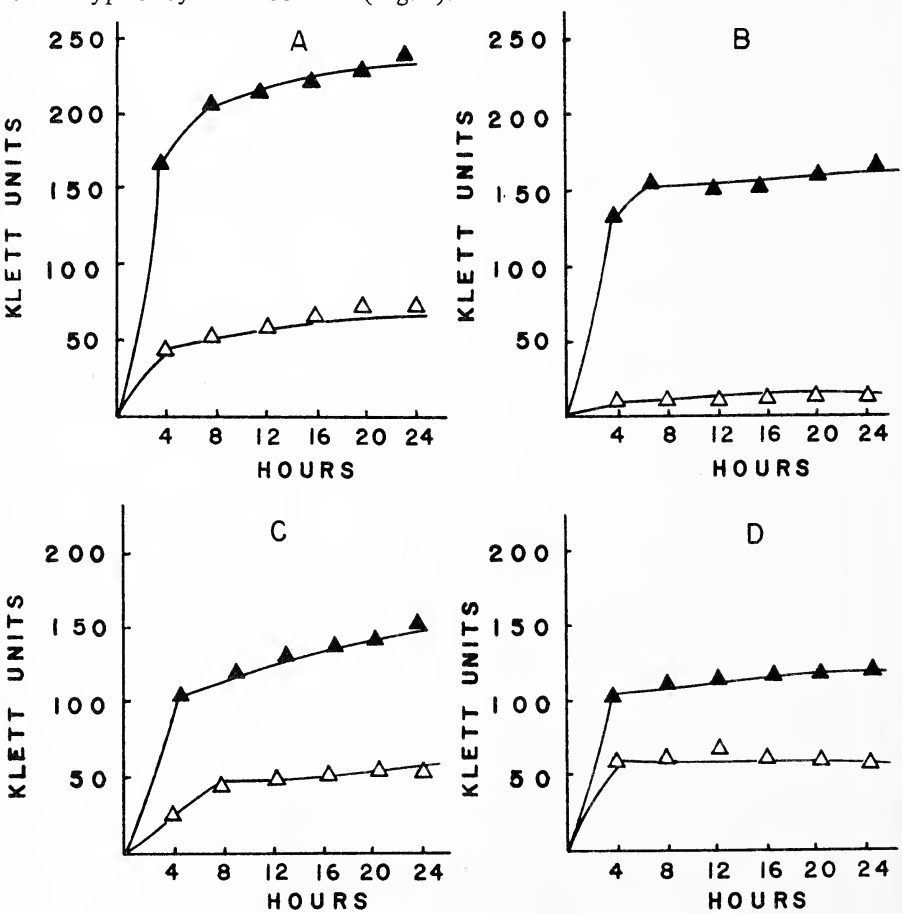


Figure 1. Growth curves of (A) *P. fluorescens*, (B) *P. vulgaris*, (C) *S. marcescens*, and (D) *E. coli* in amniotic fluid and in control tryptic soy broth. –△– amniotic fluid; –▲– tryptic soy broth.

Viable counts of the bacteria also were made on several of the dilutions of amniotic fluid which were inoculated with *E. coli* to confirm the results of the indirect method. These results are seen in Table 2.

TABLE 2

The viable count of *E. coli* grown in amniotic fluid diluted with saline.

| % of Amniotic Fluid | No. of Organisms | |
|---|---|---|
| | 12 hrs | 24 hrs |
| 10 | 8 | 10 |
| 50 | 23 | 19 |
| 80 | 92 | 78 |
| 90 | 49 | 54 |
| 100 | 23 | 18 |

*Enrichment of Amniotic Fluid*

The results of the amniotic fluid samples enriched with tryptic soy broth are found in Fig. 2. These results demonstrated that amniotic fluid retained its inhibitory

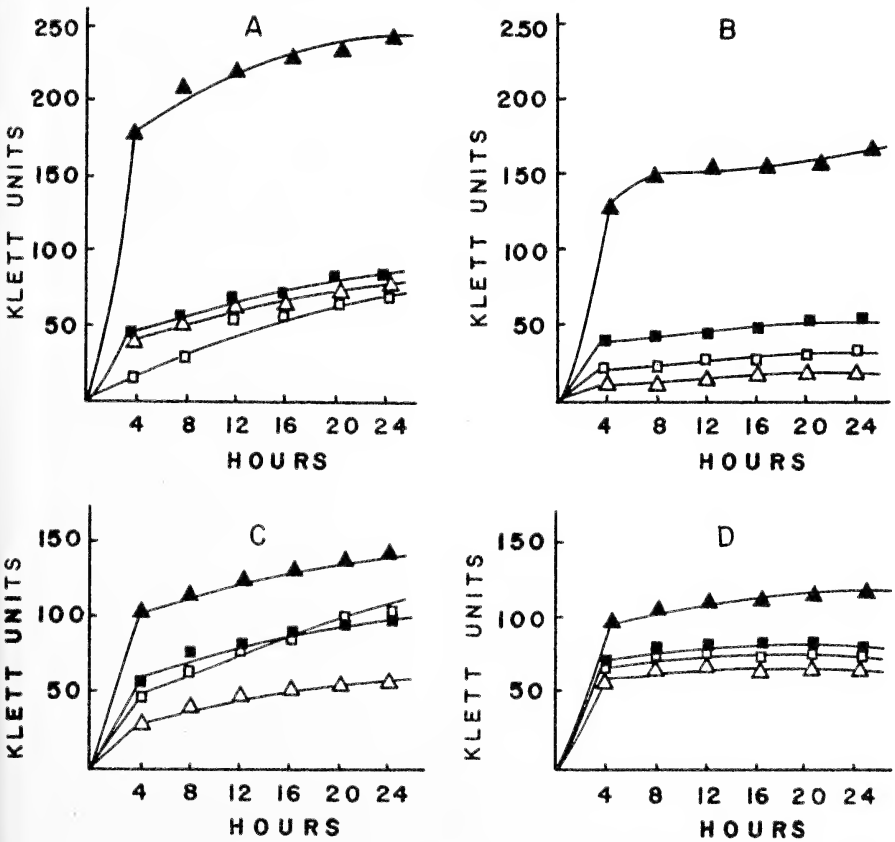


Figure 2. Growth curves of (A) *P. fluorescens*, (B) *P. vulgaris*, (C) *S. marcencens*, and (D) *E. coli* in amniotic fluid, control tryptic soy broth, and in amniotic fluid enriched with tryptic soy broth. –△– amniotic fluid; –▲– tryptic soy broth; –□– amniotic fluid plus 10% tryptic soy broth; –■– amniotic fluid plus 20% tryptic soy broth.

capacity when it was supplemented with varying amounts of tryptic soy broth. If amniotic fluid were nutritionally deficient and contained no antibacterial factors, the addition of tryptic soy broth should have resulted in better growth. The inhibitors present in amniotic fluid could be diluted out when the samples contained over 50% tryptic soy broth (Table 3). In some cultures containing mainly tryptic soy broth, enhancement of bacterial growth was observed. In these cultures the amount of inhibitor present was negligible.

TABLE 3

The effect of the enrichment of amniotic fluid with tryptic soy broth on bacterial growth.

| | NET KLETT UNIT CHANGE PER 24 HRS | | | | | |
|---|---|---|---|---|---|---|
| | % of Amniotic Fluid | | | | | |
| | 0 | 10 | 20 | 30 | 40 | 50 |
| *E. coli* | 111 | 101 | 103 | 95 | 99 | 98 |
| *Ps. fluorescens* | 244 | 194 | 187 | 174 | 160 | 143 |
| *P. vulgaris* | 157 | 164 | 162 | 154 | 139 | 124 |
| *S. marcescens* | 149 | 166 | 181 | 164 | 160 | 148 |

*Effect of the Inocula*

Culturing bacteria to be used for inoculating purposes in amniotic fluid did not prove successful. The inhibitors present in the amniotic fluid prevented sufficient growth of the organisms. With every species of bacteria studied, the amniotic fluid-grown inocula exhibited less growth than the inocula grown in tryptic soy broth (Table 4).

TABLE 4

The effect of inoculating amniotic fluid samples with amniotic fluid-grown cells.

| | | NET KLETT UNIT CHANGE PER 24 HRS | |
|---|---|---|---|
| Organism | % of Amniotic Fluid | Amniotic Fluid-Grown Cells | Control Cells |
| *E. coli* | 100 | 60 | 83 |
| *E. coli* | 50 | 38 | 50 |
| *E. coli* | 10 | 8 | 9 |
| *Ps. fluorescens* | 100 | 20 | 64 |
| *Ps. fluorescens* | 50 | 2 | 28 |
| *P. vulgaris* | 100 | 27 | 32 |
| *P. vulgaris* | 50 | 17 | 25 |
| *P. vulgaris* | 10 | 9 | 16 |

*Effect of Prolonged Incubation Time*

Extending the incubation period of the organisms studied demonstrated that amniotic fluid and dilutions thereof supported minimal bacterial growth, and within 24 hrs peak numbers of organisms were obtained. The results in Fig. 3 are those of the *E. coli* strain studied.

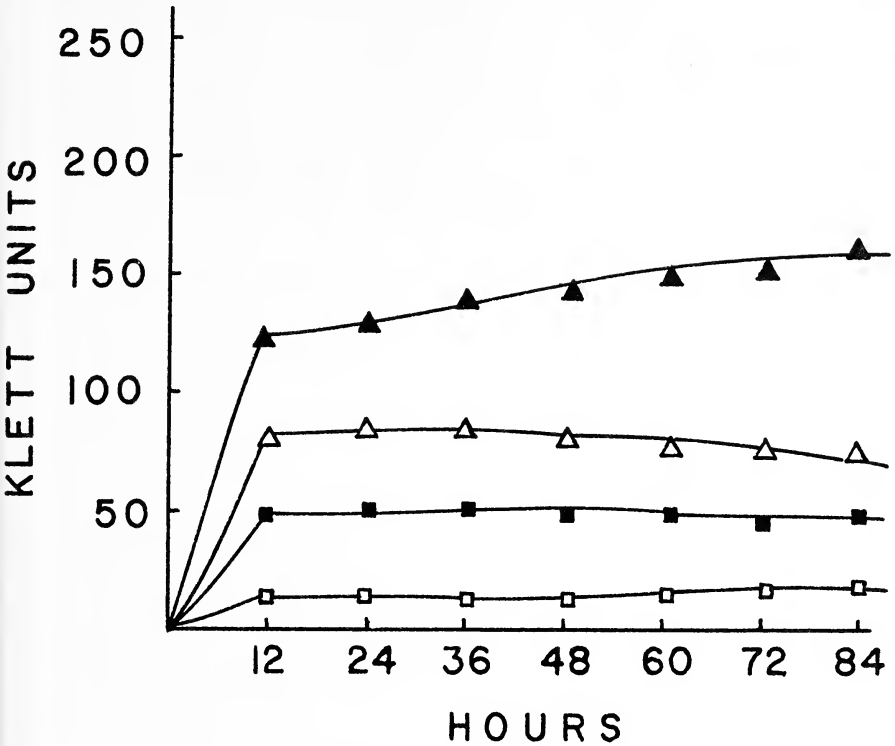


Figure 3. The effect of prolonged incubation time on the growth of *E. coli* in amniotic fluid and dilutions of amniotic fluid. –△– 100% amniotic fluid; –■– 50% amniotic fluid; –□– 10% amniotic fluid; –▲– tryptic soy broth control.

*Heat Resistance of Amniotic Fluid*

Fig. 4 shows the growth curves of *E. coli* grown in 100% amniotic fluid samples that were exposed to different temperatures. No differences are seen in these growth curves, indicating that the antibacterial factor(s) associated with amniotic fluid are quite stable to temperature extremes. Other experiments in which dilutions of amniotic fluid were used produced similar results. In each case examined the inhibitory factor(s) remained stable.

*Effect of Fetal Gestation Age on Amniotic Fluid*

The gestation age becomes an important factor when the antibacterial activity of amniotic fluid is considered. Fig. 5 shows that as the fetal age at which the amniotic fluid was collected increases, the amount of bacterial growth decreases.

*Long-Term Stability of the Amniotic Fluid Inhibitor(s)*

The effect of the storage for extended periods of time on the amniotic fluid inhibitor(s) is seen in Fig. 6. These results demonstrate that the inhibitor(s) are quite stable when refrigerated at 5 C for 15 weeks.

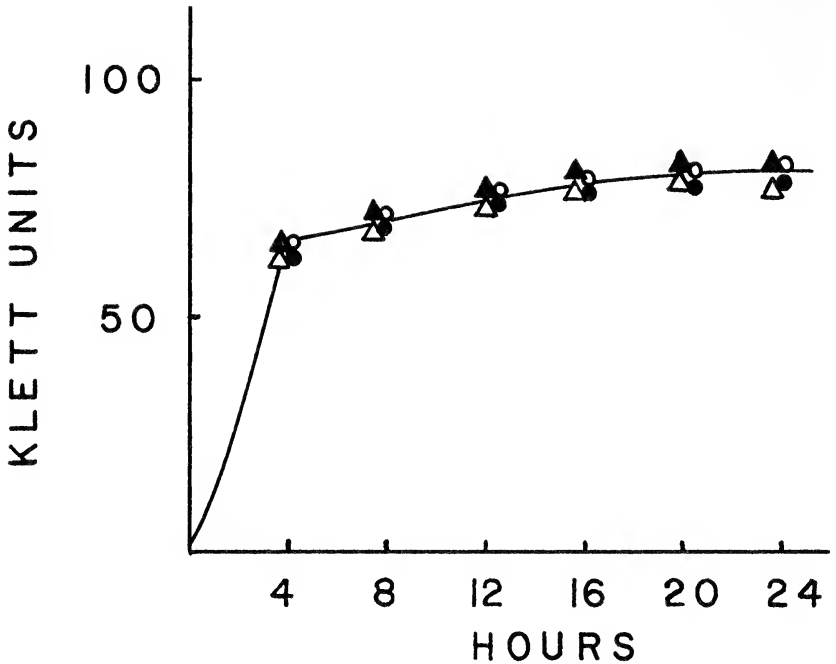


Figure 4. Growth of *E. coli* in amniotic fluid exposed to temperature variations. —●— 5 C; —▲— 100C; —○— 121 C; —△— -20 C.

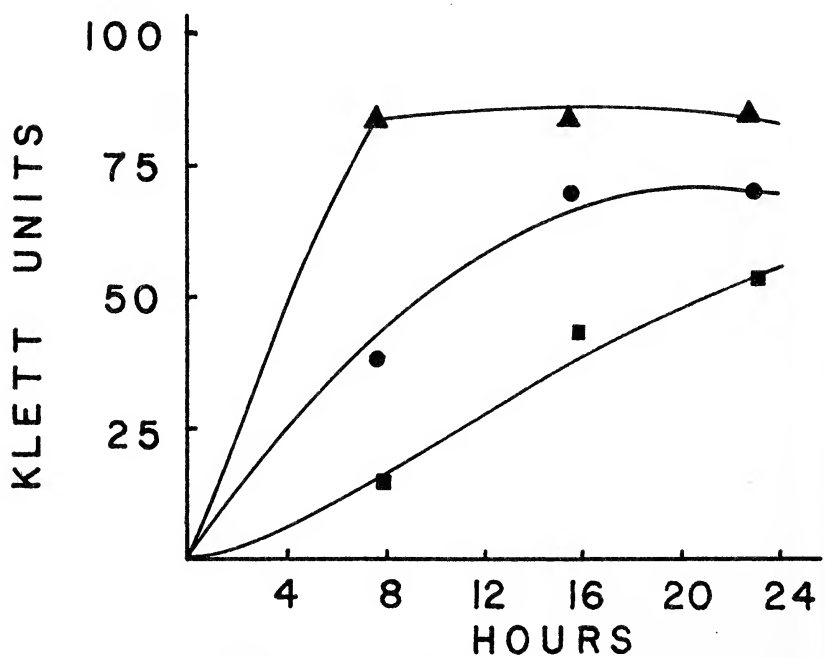


Figure 5. The effect of fetal gestation age on the growth of *E. coli* in amniotic fluid. —▲— early embryo; —●— mid-gestation fetus; —■— term fetus.

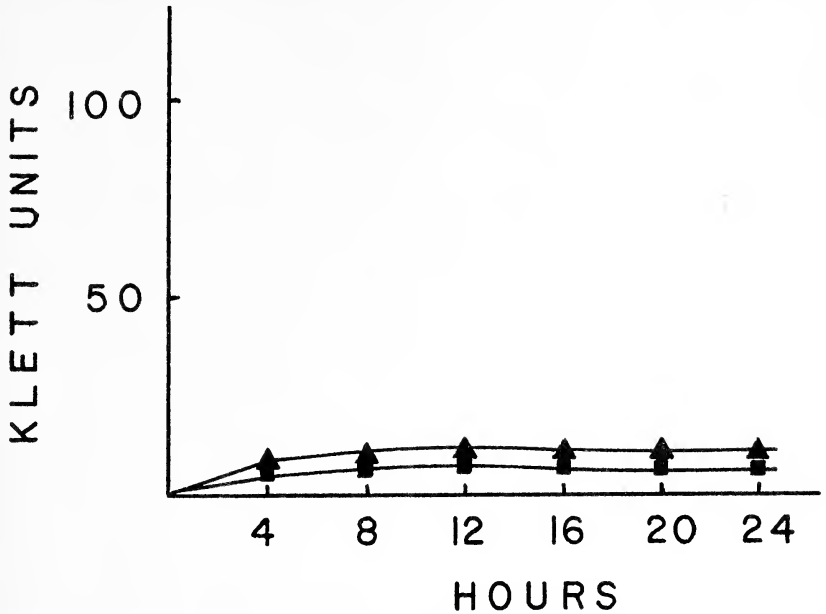


Figure 6. The long-term stability of the amniotic fluid factors in terms of their effects on the growth of *P. vulgaris*. –■– amniotic fluid stored for 24 hrs; –▲– amniotic fluid stored for 15 weeks.

## DISCUSSION AND SUMMARY

This study has shown that bovine amniotic fluid will not support the growth of bacterial species as well as control laboratory medium will and that it will only support poorly any growth at all. Dilutions made of amniotic fluid indicated that the inhibitor substance(s) could be diluted out before the loss of nutritional adequacy occurred. Moreover, the addition of nutrients to the amniotic fluid was not beneficial to supporting growth of the bacteria, presumably because of potent inhibitor(s) present in high concentrations of amniotic fluid. Two aspects of the stability of the antibacterial factor(s) were investigated and revealed that the inhibitory substance(s) remained stable when exposed to extremes of temperature and when stored over long periods of time.

Much of the controversy in the literature regarding the antibacterial activity of amniotic fluid cannot be resolved easily (Gusjdon, 1962; Walsh, *et al.*, 1965; Sarkany and Gaylarde, 1968; Galask and Snyder, 1968, 1970; Bergman, *et al.*, 1972; Cherry, *et al.*, 1973; Kitzmiller, *et al.*, 1973; Galask, *et al.*, 1974; Larsen, *et al.*, 1974a, 1974b); however, the data obtained in the course of this investigation should lend support to certain of these findings. Furthermore, the use of bovine amniotic fluid in this study circumvented some of the problems encountered by other workers who used human amniotic fluid; namely, small aliquots of amniotic fluid, contamination of the fluid with blood, and the necessity of filter sterilization.

Throughout the course of this study it was demonstrated that amniotic fluid did not support significant growth in any of the bacterial species used because of the antibacterial activity of the fluid. These results do not agree with those of other investigators who claim either that amniotic fluid will support bacterial growth or that it will not support bacterial growth due to nutritional deficiences.

ACKNOWLEDGEMENTS

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# DENTITIONAL PHENOMENA AND TOOTH REPLACEMENT IN THE SCABBARD FISH *TRICHIURUS LEPTURUS* LINNAEUS (PICES : TRICHIURIDAE)

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ABSTRACT

Dentitional phenomena and tooth replacement were analyzed in a series of Scabbard fish, *Trichiurus lepturus,* from the Gulf Coast of Texas. The dentition of this species is adapted for impaling and holding prey. Teeth in the premaxilla and dentary may be simple, compressed blades or elongate, recurved, barbed fangs. Replacement on the premaxilla involves 2 units, a medial and a lateral unit. Replacement in the medial unit involves primary and accessory alveoli at 3 tooth positions, which are occupied by fangs. Replacement teeth form in accessory alveoli and migrate to the primary alveoli where they become ankylosed. Replacement in the lateral unit of the premaxilla is typical of most predaceous polyphyodont vertebrates. The dentary has 2 replacement units, an anterior unit of 2 alveoli which are occupied alternately by fangs, and a posterior unit in which replacement operates as it does in the lateral premaxillary unit.

## INTRODUCTION

Our knowledge of tooth replacement in lower vertebrates is incomplete. In the works of Edmund (1960, 1969), the general pattern of tooth replacement was described (primarily in reptiles) as typically polyphyodont with rear to front waves of replacement throughout life.

In this paper, I analyze the dentitional phenomena in *Trichiurus lepturus,* a member of the predaceous fish family Trichiuridae.

*Trichiurus lepturus* possess a very specialized dentition characterized by fangs in the anterior portions of the premaxillary and dentary bones.

The trichiurids were reviewed taxonomically by Tucker (1956), who used only a few characters of the dentition in his classification. Tucker proposed a scheme which would include the Trichiuridae and Gempylidae as evolutionarily related scombroid fishes, with the Trichiuridae having been derived from the Gempylidae. Starks (1911) discussed the osteology of the family and included comments on the shape of the premaxillary and dentary fangs, on the occurrence of alveoli, and on the development of replacement teeth within the jaw bones.

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## MATERIALS AND METHODS

Fourteen specimens, 56 dentigerous bones, of *T. lepturus* were examined. Specimens were obtained from a fish kill in Galveston County, Texas, and are retained in my private collection.

The dentigerous bones were dissected and dried. Because of the decomposition, no sex, weight, length, or age data were taken.

Data taken were: length of the dentigerous bones, extent of regional differentiation of the teeth, number of primary and accessory alveoli, number of tooth positions, extent of embryogenesis, reabsorption and ankylosis of teeth, sequence of tooth replacement, and number of replacement waves.

Measurements were made to the nearest millimeter. Replacement teeth, and embedded portions of functional teeth were observed with a dissecting microscope with a glass insert in the base. A strong light was directed through the base upon which the bones were positioned. Due to the extremely thin bone of the jaws, even the smallest replacement teeth could be observed by this method.

Terminology for different surfaces of the teeth is that of Peyer (1968). Use of the term *fang* is a retention of that proposed by Tucker (1956). The term accessory alveolus was suggested by Edmund (1960). My interpretation of cranial osteology follows Starks' (1911).

## TOOTH BEARING BONES AND REGIONAL DIFFERENTIATION OF TEETH

### *Premaxilla*

The premaxilla contains a lateral dentitional unit of 29-36 tooth positions, and a medial dentitional unit of 3 positions which are occupied by fangs. Teeth of the medial unit are ankylosed into alveoli and those of the lateral unit are embedded in a longitudinal dental groove. Fangs are elongate, barbed teeth which curve first caudad, then recurve anteriad toward the tip (Fig. 1). Cutting surfaces are present on the entire labial border and on both the labial and lingual border of the barbed tip. The lingual border, excluding the barbed tip, is rounded.

Teeth of the lateral unit posterior to Position 10 or 11 are laterally compressed with cutting surfaces on the mesial and distal borders. Anterior to this position, the size of the teeth is abruptly reduced and the teeth are more attenuated without cutting edges. The long axis of each tooth is perpendicular to the long axis of the premaxilla up to Position 19 or 20, then it is directed somewhat caudad. Vertical height of these teeth increases to about halfway on the bone, then decreases to the end of the series.

### *Dentary*

The dentary contains a posterior unit of 18-24 positions in a longitudinal dental groove, and an anterior unit of 2 alveoli, one of which is occupied by a fang. One of the alveoli is anterior medial with respect to the other. There is a

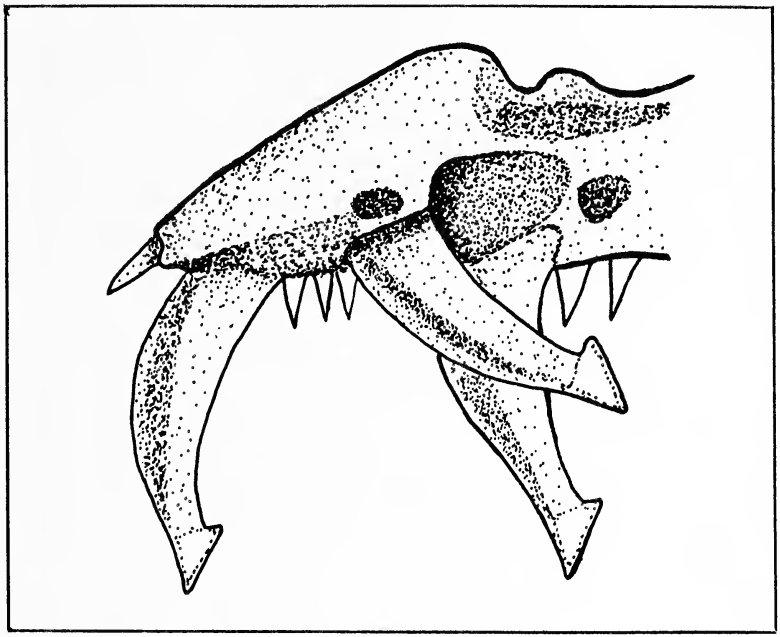

Figure 1. Anterior medial view of the right premaxilla of ECM-D-9, *Trichiurus lepturus.*

short diastema of about 5 tooth positions separating the units. Fangs of the dentary are shorter and less curved than those of the premaxilla.

Teeth of the posterior unit, in the anterior and posterior portions, are similar, although less expanded mesio-distally, to those of the lateral premaxillary unit. In the middle portions of this unit, the teeth develop barbed tips and cutting surfaces similar to those of the anterior unit. Numbers of barbed dentary teeth (0-3) seem to increase during ontogeny. No specimen with a dentary length of less than 35 mm possessed a functional barbed tooth in the posterior dentary series. Two specimens possessed 3 barbed teeth (ECM-D-8, 44 mm and ECM-D-6, 49 mm). A specimen with a dentary length of 50 mm (ECM-D-4) has one barbed dentary tooth.

## TOOTH DEVELOPMENT, ANKYLOSIS AND REABSORPTION

Tooth development begins at the tip and proceeds to the base. Ankylosis appears to be by a cementum-like substance rather than by fibrous connective tissue. Tooth development occurs within the dentigerous bones.

Reabsorption begins at the base and proceeds toward the tip, eventually attacking the ankylosing substance.

## REPLACEMENT

*Medial Premaxillary Unit*

Replacement in this unit involves a primary and an accessory alveolus at 3 tooth positions. Functional teeth are ankylosed into primary alveoli which are nearly perpendicular to the long axis of the premaxilla (Fig. 1). Replacement teeth form in an accessory alveolus which opens dorsal and posterior to the primary alveolus on the medial aspect of the bone. Replacement teeth completely form in the accessory alveolus. The fangs make an anterior-ventral migration into the primary alveolus after reabsorption of the bone separating the 2 alveoli. Reformation of the 2 alveoli occurs after the migration. Reabsorption of the functional tooth occurs simultaneously with the development of a replacement at each position. The usual sequence of replacement (20 of 28 premaxilla) appears to be in an alternating pattern. In the 8 premaxilla which do not appear to have continuous replacement, the condition is probably one of a tooth in the middle alveolus being followed by a replacement in alveolus 1 or 3. This is a typical replacement sequence which would be expected in 1/3 of the specimens. Replacement at Position 1 may proceed faster or tooth retention may be longer at that position. When right and left premaxilla of 14 specimens were considered, only 3 primary alveoli were vacant at Position 1. In 12 dentigerous bones, the primary alveoli at Position 2 were vacant, and in 9 the primary alveoli at Position 3 were vacant (Table 1). No synchrony of replacement, between right and left premaxilla, was evident in 13 of 14 specimens.

TABLE 1

A summary of the replacement phenomena in the medial premaxillary unit of *Trichiurus lepturus.*

| Tooth Condition | Position 1 | | Position 2 | | Position 3 | |
|---|---|---|---|---|---|---|
| | Right Alveolus | Left Alveolus | Right Alveolus | Left Alveolus | Right Alveolus | Left Alveolus |
| Fully ankylosed | 7 | 10 | 8 | 3 | 4 | 9 |
| Unankylosed | 1 | 0 | 0 | 1 | 2 | 0 |
| Partially ankylosed | 2 | 2 | 2 | 2 | 0 | 2 |
| Reabsorption evident | 2 | 1 | 0 | 0 | 0 | 2 |
| Socket vacant | 2 | 1 | 4 | 8 | 8 | 1 |
| Broken ankylosed | 0 | 2 | 1 | 2 | 1 | 1 |

*Dentary and Lateral Premaxillary Units*

Replacement teeth of the anterior dentary unit form deep within the alveoli. Formation of a replacement tooth begins before the loss of the old functional tooth at that position. In the right dentary of ECM-D-5 both alveoli have at least partially functional teeth. Usually, loss of an old functional tooth occurs prior to ankylosis of a replacement in the other alveolus. Each alveolus is occupied alternately without any synchrony between jaws.

The posterior dentary and lateral premaxillary units exhibit posterior to anterior replacement waves. About half the tooth positions in any dentigerous bone possess at least partially functional teeth (Table 2). Replacement waves in the dentary operate for the full length of the odd series as well as for the full length of the even series of teeth. Replacement in the premaxilla operates in 2 waves in the odd and 2 waves in the even series of teeth. The usual line of demarcation between the 2 waves is at a level lateral to the posterior edge of the third premaxillary fang. One specimen (ECM-D-10) exhibits a reversal in the direction of replacement at about tooth Position 16. Usually, about 4 generations of teeth are evident in any particular dentary or premaxilla, and no more than 2 teeth occur at any one tooth position.

TABLE 2

A summary of tooth positions and functional teeth in the lateral premaxillary unit and the posterior dentary unit of *Trichiurus lepturus*.

| | Functional Teeth | | Tooth Positions | |
|---|---|---|---|---|
| Bone | Range | Mean | Range | Mean |
| Premaxilla | 13-18 | 15.2 | 29-36 | 31.1 |
| Dentary | 8-11 | 9.9 | 18-24 | 22.2 |

I have chosen the right dentary of ECM-D-9 to use as an example here (Fig. 2). Four generations of teeth, in rear to front waves are evident, when the exposed height of each tooth is considered.

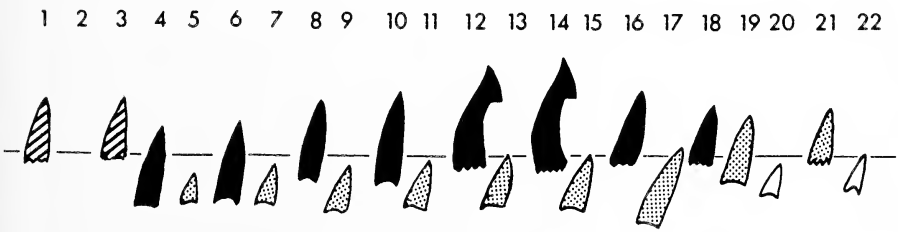


Figure 2. Semischematic representation of the replacement sequence in the posterior unit of the right dentary of ECM-D-9, *Trichiurus lepturus*. Tooth generation 1 is represented by diagonal lines, 2 by solid symbols, 3 by stippling, and 4 by open symbols. The horizontal line between teeth indicates the jaw line, and numbers indicate tooth positions.

## DISCUSSION

Development of the cutting surfaces on the labial border of the fangs and the presence of barbed tips are probably adaptations toward penetration and holding

rather than shredding of tissue, since it would seem likely that a tooth adapted for shredding would possess a greater cutting surface on the lingual aspect. The greater mesio-distal expansion of the lateral premaxillary dentition may retard penetration. These teeth may function in holding the prey in a fixed position while the upper jaw forces the prey down onto the barbed teeth of the posterior dentary series. Evidence that the number of barbed dentary teeth increases during ontogeny may reflect a change in feeding habits with age. The reduced size of the teeth in the anterior end of the lateral premaxillary unit and their position, which is well removed from the occlusal surfaces of the premaxillary fangs which they flank, may indicate that these teeth do not function in the feeding activities of this fish.

The development of each replacement tooth occurs in a manner well documented (Bogert, 1943; Edmund, 1960, 1969; Peyer, 1968). Development of teeth within the jaw bones is an adaptation for the protection of growing teeth from being dislodged by a struggling prey. Similar development of teeth occurs in predaceous fish such as *Lepidotes* (Peyer, 1968) and most of the characoid genera (Roberts, 1967). Reabsorption of teeth occurs in a manner similar to other polyphyodont vertebrates (Edmund, 1960, 1969; Peyer, 1968).

The occurrence of accessory alveoli well away from the occlusal surfaces of the functional premaxillary teeth, seems to be an adaptation for predation. Accessory alveoli have been reported by Morgan (1973) in the sibynophiine genus *Scaphiodontophis;* Edmund (1960) in ornithiscian dinosaurs; and by Roberts (1967) in the characoid genera *Hydrocynus* and *Alestes.*

Longer tooth retention or more rapid tooth replacement at Position 1 in the premaxilla would be a selective advantage in a predaceous animal since it is this tooth position which contacts the prey first. Asynchrony between right and left premaxillary units probably maintains at least 1 functional tooth at 3 levels in the series when both premaxilla are considered as a functional unit. If longer tooth retention is a fact, then there will often be 2 teeth at alveolus 1, due to an overlap in the replacement sequence between right and left premaxilla.

The replacement sequence on the dentary and lateral premaxillary units is typical of polyphyodont vertebrates (Bogert, 1943; Edmund, 1960, 1969; Peyer, 1968). The low number of replacement teeth suggests that replacement may be slow. The holostean *Lepisosteus,* with intense replacement, exhibits a high number of replacement teeth (Peyer, 1968). The condition in *T. lepturus* may be feasible because of the well-protected position of the replacement teeth, the deep implantation of the functional teeth, and the cementum type of ankylosing substance.

## CONCLUSIONS

The dentition of *T. lepturus* is adapted for impaling and holding prey. Teeth in the premaxilla and dentary are either laterally compressed blades or elongate, recurved, barbed fangs. Replacement on the premaxilla involves primary and

accessory alveoli at 3 tooth positions, which are occupied by fangs. Replacement teeth form in accessory alveoli and migrate to the primary alveoli where they become ankylosed. Replacement in the lateral unit of the premaxilla is typical of most predaceous polyphyodont vertebrates. The dentary has 2 replacement units, an anterior unit of 2 alveoli which are occupied alternately by fangs, and a posterior unit in which replacement operates as it does in the lateral premaxillary unit.

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# EVIDENCE FOR THE SPECIES STATUS OF BAIRD'S RATSNAKE

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## INTRODUCTION

When allopatric populations of similar taxa have ranges which narrowly overlap geographically, the systematist must decide whether he is dealing with subspecies of the same species or with 2 species. Mayr, *et al.*, (1953) in review of the characteristics of allopatric populations with narrow zones of overlap, showed that in those cases where there is complete interbreeding over relatively broad or very narrow zone of contact, one is dealing with subspecies of the same species, but where only occasional hybrids or no interbreeding occur at the zone of contact, one is dealing with different species.

In 1880, Yarrow (in Cope) described *Coluber bairdi* from Ft. Davis, Jeff Davis County, Texas. Dowling (1951), citing only 2 specimens of *bairdi*, but alluding to others, referred it to a subspecies of *Elaphe obsoleta.* He argued that although *bairdi* has more subcaudals and dorsal blotches, those same features in *obsoleta* increase in number towards the west; he envisioned *bairdi* as representing the western extremes of clinal variation, stating that a few Kerr County specimens represented an intergrade population on the basis of dorsal blotch number.

Wright and Wright (1957) treated *Elaphe bairdi* as a species, mentioning that it could be separated from *Elaphe obsoleta* by its usually greater number of subcaudals and dorsal blotches.

The present study is based on specimens of *Elaphe bairdi* from throughout its known range, and of *Elaphe obsoleta* from nearby localities in central Texas. Specimens have been examined from the following collections: Chicago Academy of Sciences; Strecker Museum, Baylor University, Waco, Texas; Sul Ross State University, Alpine, Texas; Texas Cooperative Wildlife Collection, Texas A&M University, College Station; Texas Natural History Collection, University of Texas, Austin; and the University of Michigan Museum of Zoology, Ann Arbor.

## DIAGNOSTIC CHARACTERS

Several characters have been used to separate *Elaphe bairdi* from *Elaphe obsoleta.* Not all of those are reliable, while others that have not been previously mentioned

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are found to be distinctive, the most useful characters being ventral counts, subcaudal counts, and pattern.

*Ventrals*

In the specimens examined, ventral counts for both sexes of *Elaphe bairdi* average distinctly higher than in adjacent populations of *Elaphe obsoleta.* Eleven male *bairdi* have 233-260 ( X = 247.8) ventrals, whereas 11 male *obsoleta* have 220-232 ( X = 225.6). The one example with 233 ventrals (other male *bairdi* have no fewer than 242) is from southern Brewster County, Texas. Nine *bairdi* females have 235-247 ( X = 241.9) ventrals, whereas those counts in 9 *obsoleta* females are 223-238 ( X = 229.0).

Males of *Elaphe bairdi* have a greater mean number of ventrals than do females, but in central Texas, ventral counts for female *Elaphe obsoleta* slightly exceed those for males. Ventral counts of female *obsoleta* from Kansas (Smith, 1956) and Illinois (Smith, 1961) also exceed those of males.

*Subcaudals*

Although some overlap is exhibited in subcaudal number, the counts average distinctly higher in *Elaphe bairdi* than in *Elaphe obsoleta.* In *bairdi,* subcaudal counts are 88-100 ( X = 94.0) in 11 males, and 82-93 ( X = 88.6) in 8 females. The counts for *obsoleta* are 75-89 ( X = 81.8) in 12 males, and 72-79 ( X = 74.0) in 7 females.

*Pattern*

A comparison of color pattern presents difficulties because of the extreme ontogenetic variation exhibited by *Elaphe bairdi,* and also because subadult and adult specimens tend to fade in preservative, revealing blotching that is masked in life. By contrast, central Texas *Elaphe obsoleta* change relatively little in pattern, except that the ground color occasionally becomes suffused with black, obscuring or almost obliterating the blotches.

Dorsal and lateral blotches are apparent only in juvenile *Elaphe bairdi,* but they remain visible throughout life in central Texas *Elaphe obsoleta* (Fig. 1). Dial (1965) illustrated the juvenile pattern of *bairdi.* Dorsal body blotches are 44-61 ( X = 50.5) in 8 *bairdi,* and 27-37 ( X = 32.0) in 20 *obsoleta.* Dorsal and lateral blotches in *obsoleta* are conspicuously longer than are those in *bairdi.* In *obsoleta* the dorsal blotches are 4-6 scales long, the lateral blotches 4-5 scales long. In *bairdi,* when blotches are present, they extend 2-2.5 scales, rarely 3, and lateral blotches 1.5-2 scales. Dorsal blotches in *obsoleta* are typically broadly H-shaped, usually transversely oblong in *bairdi.*

In *Elaphe bairdi,* the blotched dorsal pattern of the juvenile gives way to the dorsolateral and lateral dark stripes of the young adult. The striped snake has a gray ground color, each scale being orange anteriorly in slightly older individuals. Later the stripes disappear, and the ground color becomes orange, deepening

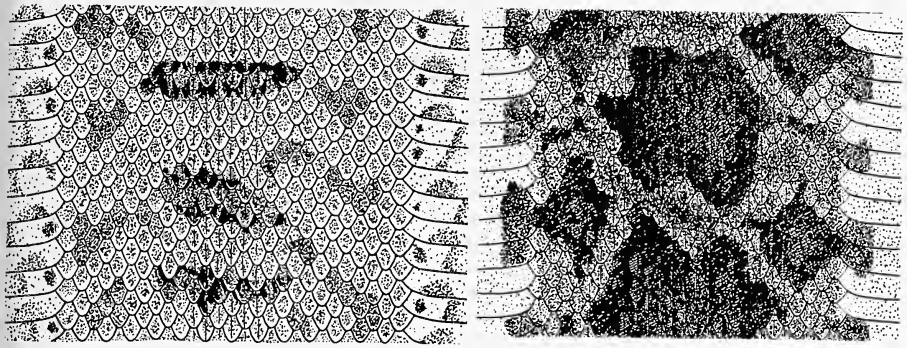

Figure 1. The midbody patterns of the juvenile *Elaphe bairdi* from Real County, Texas (left), and of an adult *E. obsoleta* from west-central Bexar County, Texas (right).

posteriorly to red-orange (Olson, 1967). The posterior 2/3 of each dorsal scale is suffused with black peppering, so that larger snakes are grayish orange in appearance.

Freshly preserved specimens (1370-1480 mm) were orange cast with faint striping; a 1571 mm male was completely without pattern in life (Olson, 1967), though, after 7 years in preservative, very faint blotch outlines are discernible. Assuming that the sexes are similar in pattern ontogeny, the juvenile pattern persists in animals about 700-800 mm long, the young adult pattern in the size range between 880-1200 mm, and adult pattern appears in snakes more than 1250 mm. In central Texas *Elaphe obsoleta* the pattern of dorsal and lateral blotches remains conspicuous throughout life.

Adults of *Elaphe obsoleta* have a uniformly black head dorsally and laterally with the exception of the lower parts of the supralabials (Fig. 2). In contrast, the sides of the head are light in *Elaphe bairdi,* only the dorsal head plates being dark gray or gray-brown (Fig. 3).

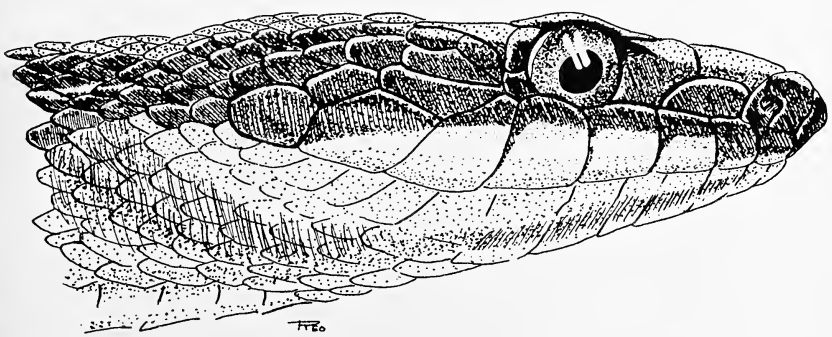

Figure 2. Lateral view of the head of an adult male *Elaphe obsoleta* (WMM 650) from south of Kerrville, Kerr County, Texas.

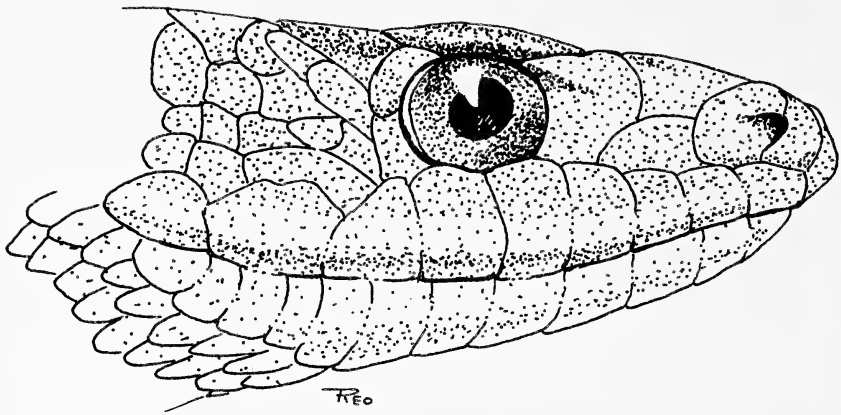

Figure 3. Lateral view of the head of an adult male *Elaphe bairdi* (REO 687) from near Alpine, Brewster County, Texas.

Other characters which have been utilized to separate the snakes taxonomically are felt to be unreliable: genials, posterior scale row reduction, relative tail length, and number of supralabials.

*Genials*

Wright and Wright (1957) mentioned the divided posterior genials in *Elaphe bairdi,* a condition not seen in *Elaphe obsoleta.* The character is variable in the series examined. A few specimens of *bairdi* have one or both posterior genials divided; many have undivided scales. Briefly, the genial condition seems constant in *obsoleta,* variable in *bairdi.*

*Posterior Scale-Row Reduction*

Reductions to 19 scale rows occurs in 90% of the *Elaphe bairdi* examined, but only 40% of central Texas *obsoleta,* the others having 21.

*Relative Tail Length*

*Elaphe bairdi* has a proportionately longer tail than does *Elaphe obsoleta.* Male *bairdi* have tail/total length values of 18.6-23.0% ( X = 20.6%), female *bairdi* 18.3-21.8% (X = 19.8%), male *obsoleta* 16.0-19.5% ( X = 17.9%), and female *obsoleta* 16.0-19.2% ( X = 17.5%).

*Supralabials*

Supralabial counts have been used as a basis for distinguishing these snakes, but are unreliable. Milstead, *et al.,* (1950) suggested that *bairdi* might some day be found to be a subspecies of *Elaphe obsoleta* on the basis of specimens of *bairdi* with 8 supralabials.

In central Texas, 92% of the *Elaphe obsoleta* examined have 8 supralabial scales on each side. However, *Elaphe bairdi* may possess 8 or 9 supralabials, or a combination thereof (Table 1).

TABLE 1

Frequency distribution of supralabial number in *Elaphe bairdi.*

| Number of Supralabials[1] | | |
|---|---|---|
| 16 | 6 | 4 |
| 17 | 2 | 1 |
| 18 | 2 | 3 |

[1]Combined count for both sides of head.

## DISCUSSION

Differences between *Elaphe bairdi* and *Elaphe obsoleta* are not diminished by geographic proximity. Such would not be expected in an intergrade situation. I am aware of 2 areas where the ranges of these forms overlap; south of Kerrville, and at Ingram, in Kerr County, Texas. A third possibility exists along the eastern edge of Medina County, but only a striped female *Elaphe bairdi* is extant from there. (Specimens of *Elaphe obsoleta* are available from 5 mi east of Medina Lake in Bexar County.)

The series from south of Kerrville includes 5 *Elaphe bairdi* (2 males, 3 females), and 2 male *Elaphe obsoleta.* The female *bairdi* have 235, 242, and 243 ventrals, 86+, 89, 90 subcaudals, and 21, 19, 19 scale rows posteriorly, while the males have 248, 250 ventrals, 89+, 100 subcaudals, and 19 scale rows posteriorly. The *Elaphe obsoleta* have 225, 229 ventrals, 89, 88 subcaudals, and 21 scale rows posteriorly. These *obsoleta* are adults with prominent blotches (30, 33) and with head uniformly dark above the lower portions of the supralabials. All *bairdi,* on the other hand, show the typical body and head patterns previously described for that form. Thus, no hybridization in apparent in that area.

From Ingram, 5 adult and subadult males are available. Of those, two are typical of *obsoleta,* having 230, 231 ventrals, 83, 83 subcaudals, 21, 19 posterior scale rows, and 34, 32 dark dorsal body blotches. Three show what is regarded to be intermediacy. One (UMMZ 102843) has 225 ventrals and 84 subcaudals as in *obsoleta,* but 44 blotches arranged in a pattern resembling the condition in juvenile *bairdi.* The other 2 hybrids (UMMZ 102844, 102845) have patterns similar to that of *obsoleta,* with 239, 235 ventrals, 91, 81 subcaudals, and 40, 42 dorsal blotches. All of these specimens have 19 posterior scale rows.

The question of whether one is dealing with a species or with subspecies in this case can be viewed with regard to 3 features of the population: the presence of an area of overlap of ranges, the nature of the population in the area(s) of overlap, and the nature of populations adjacent to the areas of overlap.

Two known areas of overlap of ranges have been mentioned above. Were intergradation occuring between subspecies of the same species, it would be expected that the entire population within the area of range overlap would reflect intermediacy in many or all of the characters. Such is not the case here. Specimens from the same localities have all characters of either *Elaphe obsoleta* or *Elaphe bairdi* or only some show intermediacy (at Ingram). This reflects a hybrid situation, not intergradation.

The populations adjacent to the areas of range overlap differ from each other 100% in characters regarded to be reliable. They do not reflect intermediacy. Again this reflects species, not subspecies status.

Thus, although some hybridization probably occurs at Ingram, it is unknown elsewhere where the ranges meet, nor is there evidence of introgression. I feel that *Elaphe bairdi* and *Elaphe obsoleta* are acting as good species, and should be so regarded.

ACKNOWLEDGEMENTS

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# ANTIGENIC ANALYSIS OF THE GENUS *AEROMONAS*

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ABSTRACT

Particulate and soluble heat stable antigens were prepared from autoclaved cultures of the recognized species and subspecies of the genus *Aeromonas* grown on solid media. Antisera were prepared in New Zealand white rabbits. Cross reactions and cross adsorptions were performed in attempts to show relationships between the members of the genus.

There was a relationship between *A. liquefaciens, A. punctata, A. punctata caviae* and *A. hydrophila* when agglutinins were demonstrated in antisera prepared with whole cells and a cell-free supernatant. In addition to species-specific antigens, the analysis showed an antigen that was shared by *A. liquefaciens, A. punctata,* and *A. punctata caviae.* In addition, *A. liquifaciens* also shared another antigen with *A. hydrophila* and yet another with *A. punctata.*

## INTRODUCTION

The genus *Aeromonas* has been known and studied for the past 80 years. Still, its classification is not clear. Several investigators have used serological methods in an attempt to classify these organisms. However, their studies did not include all of the species and subspecies in this genus. Guthrie and Hichner (1943), when working with strains of *A. hydrophila,* found them to constitute an antigenic heterogeneous group. Miles and Miles (1951) also showed antigenic heterogeity when testing 12 strains from different sources and antisera to 7 of them. Kjem (1955) carried out cross agglutination experiments with 5 *Aeromonas* strains using O (Somatic) antigens and H (flagella) antigen preparations. He found 2 of these strains had O related antigens but were otherwise unrelated. Alodova and Geizer (1968) worked with 14 strains of *A. shigelloides.* Cross O-agglutination showed that most of the strains had a distinct O-antigen or O-antigen complex. Three strains proved to be more related to each other than to the remaining strains examined. In H-antigen cross agglutinations, they found 5 groups having specific antigens for their group, one of which could be divided into subgroups. Ewing, *et al.,* (1961), using several strains of *Aeromonas* from the cultural collection of CDC-RH, established 12 previsional O-antigen groups and 9 H-antigen groups and differentiated a number of serotypes within each group. Among the interrelationships between species shown by them include antigens shared by the following 5 pairs: *A. salmonicida* and *A. hydrophila; A. formicans* and *A. liquefaciens;*

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*A. formicans* and *A. shigelloides; A. hydrophila* and *A. formicans;* and *A. shigelloides* and *A. punctata.*

This paper reports the antigenic relatedness between all available species and subspecies of *Aeromonas* with the use of 2 heat stable somatic antigen preparations.

## METHODS AND MATERIALS

### *Antigens*

Representatives of available species and subspecies of the genus *Aeromonas* were used in this study. These included: *A. hydrophila* ATCC 0971, *A. hydrophila formicans* ATCC 13137, *A. liquefaciens* ATCC 14715, *A. proteolytica* ATCC 15338, *A. punctata* ATCC 11163, *A. punctata caviae* ATCC 14486, *A. salmonicida* # 61 Italy MS-320 West Fish Disease Lab, and *A. shigelloides* ATCC 14029.

Excepting *A. salmonicida* and *A. proteolytica,* all cultures for antigen preparation were grown in Brain Heart Infusion (BHI) Broth (Difco) starter cultures which were used to inoculate BHI agar in Roux bottles. Growth was for 24 hrs at 37 C. *A. proteolytica* requires sea water for growth (Merkel, *et al.,* 1964) and consequently was grown under the above conditions except on 2% casitone made with artificial sea water. *A. salmonicida* requires a growth temperature of 22 C. After incubation, the cells were harvested in saline in a flask containing glass beads and agitated on a reciprocal shaker for 1 hr. After separation, the cells were washed in saline and killed by autoclaving at 15 lb psi and 121 C. The heat-killed suspension was then centrifuged and the supernatant filtered. Both the supernatant (soluble antigen) and suspended cells ($6 \times 10^6$) (particulate antigen) were refrigerated at 4 C.

### *Antiserum*

Two types of antisera were prepared against the described strains. One type was directed against the particulate antigen and the other against the soluble supernatant antigens. New Zealand white rabbits of mixed sex and weighing 2-3 kg were pre-bled and tested for natural antibody. The antigens were mixed with Freund's (1957) incomplete adjuvant in 1:1 proportions and 2 ml of this material given in each hip once a week for 2 weeks. On the third week, the animals were given 2 ml of 1:1 mixture of antigen and Freund's complete adjuvant.

### *Serological Cross Reactions*

A tube agglutination test, utilizing both homologous and heterologous serological systems were used to assess the antigenic relatedness of the various species. Two-fold dilutions of antiserum were prepared. The saline used for dilution contained 2% gelatin to minimize auto-agglutination. Incubation was at 56 C for 5-6 hrs (Kabjt and Meyer, 1948) followed by overnight refrigeration.

*Agglutinin Adsorption and Antigenic Analysis*

Adsorption studies were conducted as dictated by results observed in the serological cross reactions. The serum was diluted 1:10 and mixed with heavy suspensions of appropriate cells. This mixture was incubated in the 56 C water bath for 4 to 5 hrs without shaking. After overnight refrigeration, the mixture was centrifuged and the clear supernatant removed. The adsorbed serum was tested for homologous and heterologous agglutination using the above procedure.

## RESULTS AND DISCUSSION

*Relationship of Particulate Antigens*

Agglutination tests were used to analyze the homologous as well as heterologous antigen-antibody systems. Cross agglutinations of the heat stable particulate systems showed that most of the strains have distinct O-antigens or O-antigen complexes. The O-antigenic relationship was only observed within 4 groups as seen in Table 1. Low titer reciprocal agglutination reactions were observed in the heterologous system of *A. salmonicida* and *A. liquefaciens,* but these were not considered significant. Some organisms reacted only in one system but not in the reciprocal (Table 1). The nature of the O-antigenic relationship between *A. liquefaciens, A. hydrophila, A. punctata* and *A. punctata caviae* is seen in Table 2. According to these adsorption experiments, *A. liquefaciens* shares an antigenic component with both *A. punctata* and *A. punctata caviae* and another in common with *A. hydrophila,* but lacked a distinct O-antigen. *A. punctata* was shown to have in addition to the antigen shared with *A. liquefaciens,* a species specific antigen. *A. punctata caviae* also had a distinct antigen in addition to one shared with *A. punctata* and *A. liquefaciens. A. hydrophila* was also found to have a distinct antigen in addition to the one shared with *A. liquefaciens.*

*Relationship with Soluble Antigens*

Homologous and heterologous relationships of antisera to soluble antigens were analyzed by reacting antisera with the various particulate (whole heat-killed cells) antigens. The results are presented in Table 3. It seems that soluble antigenic complexes of *A. liquefaciens, A. punctata* and *A. punctata caviae* are capable of stimulating antibodies to related antigens, thus share common antigens, whereas *A. hydrophila, A. hydrophila formicans, A. proteolytica, A. salmonicida* and *A. shigelloides* possess unique antigens since the latter organisms did not stimulate cross reacting antibodies. The relatedness of *A. liquefaciens, A. punctata* and *A. punctata caviae* as determined by cross adsorption experiments is seen in Table 4. Again, *A. liquefaciens* was found to share an antigen with *A. punctata* and *A. punctata caviae.* However, the adsorptions also indicate that *A. liquefaciens* does have a distinct antigen and an antigen shared with *A. punctata* in addition to the antigen shared with *A. punctata* and *A. punctata caviae.* Adsorptions with *A. punctata* verified the presence of these antigens and also its

type specific antigen. As before, studies with *A. punctata caviae* showed the presence of antigen shared with *A. liquefaciens* and the species-specific antigen.

TABLE 1

Serological Cross Reactions: Antisera to Particulate Antigens

| Serum | Antigen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *A. hydrophila* | *A. hydrophila formicans* | *A. liquefaciens* | *A. proteolytica* | *A. punctata* | *A. punctata caviae* | *A. salmonicida* | *A. shigelloides* |
| *A. hydrophila* | 1:160 | 0 | 1:40 | 0 | 0 | 0 | 0 | 0 |
| *A. hydrophila formicans* | 0 | 1:160 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. liquefaciens* | 1:40 | 0 | 1:160 | 1:20 | 1:40 | 1:20 | 1:10 | 0 |
| *A. proteolytica* | 0 | 0 | 0 | 1:160 | 0 | 0 | 0 | 0 |
| *A. punctata* | 0 | 0 | 1:40 | 1:20 | 1:160 | 1:40 | 1:10 | 1:20 |
| *A. punctata caviae* | 0 | 0 | 1:40 | 1:20 | 1:40 | 1:160 | 0 | 1:40 |
| *A. salmonicida* | 0 | 0 | 1:10 | 0 | 0 | 0 | 1:160 | 0 |
| *A. shigelloides* | 1:20 | 0 | 1:10 | 0 | 0 | 0 | 0 | 1:160 |

TABLE 2

Cross Adsorptions: Antisera to Particulate Antigens

| Serum | Adsorbed With | Reacted With | | | |
|---|---|---|---|---|---|
| | | *A. hydrophila* | *A. liquefaciens* | *A. punctata* | *A. punctata caviae* |
| *A. liquefaciens* | | 1:40 | 1:160 | 1:40 | 1:20 |
| | *A. hydrophila* | 0 | 1:40 | 0 | 0 |
| | *A. punctata* | 1:20 | 1:40 | 0 | 0 |
| | *A. punctata caviae* | 1:20 | 1:40 | 0 | 0 |
| *A. punctata* | | 0 | 1:40 | 1:160 | 1:40 |
| | *A. liquefaciens* | 0 | 0 | 1:40 | 0 |
| | *A. punctata caviae* | 0 | 0 | 1:40 | 0 |
| *A. punctata caviae* | | 0 | 1:40 | 1:40 | 1:160 |
| | *A. liquefaciens* | 0 | 0 | 0 | 1:40 |
| | *A. punctata* | 0 | 0 | 0 | 1:40 |
| *A. hydrophila* | | 1:160 | 1:40 | 0 | 0 |
| | *A. liquefaciens* | 1:20 | 0 | 0 | 0 |

Unlike in the particulate antigen adsorptions, there was no relationship demonstrated between *A. liquefaciens* and *A. hydrophila.* In the 8 strains studied, each had a distinct species-specific O-antigen or O-antigenic complex and in addition, 4 had interrelated O-antigens. They were: *A. liquefaciens, A. punctata, A. punctata caviae,* and *A. hydrophila.* However, some difference existed between the 2 analyses. In the particulate antigenic analysis, the relationship between *A. hydrophila* and *A. liquefaciens* was observed, a relationship that was not observed in the soluble antigen analysis.

In the particulate antigenic analysis, a specific antigen to *A. liquefaciens* and a common antigen between *A. liquefaciens* and *A. punctata* were not demonstrated, whereas in the soluble antigen analysis, they were observed.

TABLE 3

Serological Cross Reactions: Antisera to Soluble Antigens

| Serum | Antigen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *A. hydrophila* | *A. hydrophila formicans* | *A. liquefaciens* | *A. proteolytica* | *A. punctata* | *A. punctata caviae* | *A. salmonicida* | *A. shigelloides* |
| *A. hydrophila* | 1:160 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. hydrophila formicans* | 0 | 1:160 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. liquefaciens* | 0 | 0 | 1:160 | 0 | 1:80 | 1:40 | 0 | 0 |
| *A. proteolytica* | 0 | 0 | 0 | 1:80 | 0 | 0 | 0 | 0 |
| *A. punctata* | 0 | 0 | 1:40 | 0 | 1:160 | 1:20 | 0 | 0 |
| *A. punctata caviae* | 0 | 0 | 1:20 | 0 | 1:20 | 1:160 | 0 | 0 |
| *A. salmonicida* | 0 | 0 | 0 | 0 | 0 | 0 | 1:160 | 0 |
| *A. shigelloides* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1:320 |

TABLE 4

Cross Adsorptions: Antisera to Soluble Antigen

| Serum | Adsorbed With | Reacted With | | |
|---|---|---|---|---|
| | | *A. liquefaciens* | *A. punctata* | *A. punctata caviae* |
| *A. liquefaciens* | | 1:160 | 1:80 | 1:40 |
| | *A. punctata* | 1:20 | 0 | 0 |
| | *A. punctata caviae* | 1:40 | 1:20 | 0 |
| *A. punctata* | | 1:40 | 1:160 | 1:20 |
| | *A. liquefaciens* | 0 | 1:40 | 0 |
| | *A. punctata caviae* | 1:20 | 1:40 | 0 |
| *A. punctata caviae* | | 1:20 | 1:20 | 1:160 |
| | *A. liquefaciens* | 0 | 0 | 1:40 |
| | *A. punctata* | 0 | 0 | 1:40 |

The analysis recorded here should be regarded only as an indication of the relationship between the recognized species and subspecies. Inclusion of more strains from each *Aeromonas* group would reveal a more complete antigenic relationship.

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# GROWTH RESPONSES OF *TILAPIA AUREA* TO FEED SUPPLEMENTED WITH DRIED POULTRY WASTE

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ABSTRACT

Blue tilapia, *Tilapia aurea,* fed for a period of 10 wks at 3% of body weight daily on a commercial trout diet control and the trout diet supplemented at 10, 20 and 30% with dried poultry waste (DPW), demonstrated decreasing rates of growth as a function of increasing DPW levels although the differences were not significant ($P > 0.10$). Food conversions for fish fed the control and 10% DPW-supplemented diets were similar (1.02 and 1.06, respectively). In a second, 8-wk experiment, *T. aurea* fed trout feed supplemented with 20% DPW failed to grow when offered feed at 1% of body weight daily. Fish fed the 20% DPW-supplemented diet at 5% daily grew at approximately the same rate as those fed 3% of that diet in the initial experiment.

## INTRODUCTION

The suitability for aquaculture of various species of *Tilapia* (family Cichlidae) has been demonstrated in many parts of the world (Chimits, 1955, 1957; Kirk, 1972; Fryer and Iles, 1972), but only recently have *Tilapia* spp. been considered as aquaculture candidates in the United States. *Tilapia* spp. tolerate relatively high temperatures (Allanson and Noble, 1964), low dissolved oxygen (Denzer, 1968; Fryer and Iles, 1972), a wide range of salinity (Lotan, 1960; Chervinski, 1961, 1966; Chervinski and Zorn, 1974) and high levels of ammonia (Stickney, *et al., In Press*).

Dried poultry waste (DPW) has been used to feed a variety of terrestrial animals including poultry, ruminants and swine (Couch, 1972). Six states have approved the use of DPW in livestock feed as of March, 1976, although the federal government has not cleared DPW in the feed of animals used in interstate commerce. Channel catfish have been successfully reared on diets heavily supplemented with DPW (Joe Lock, personal communication).

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This research represents preliminary studies to determine the feasibility of supplementing tilapia feed with DPW, and was supported, in part, by Project HM-2831 of the Texas Agricultural Experiment Station, Texas A&M University. *Tilapia aurea,* the blue tilapia, was chosen for study owing to its presence in Texas in several power plant cooling reservoirs.

## MATERIALS AND METHODS

Two experiments were conducted indoors at the Aquaculture Research Center of the Texas Agricultural Experiment Station, near College Station, Texas. Aluminum troughs (3.05 m X 51 cm) containing water 20 cm in depth were each stocked with 100 *T. aurea* averaging 5.5 g. Individual fish weights were not obtained; however, the fish were of approximately the same age and were selected to be of uniform size. Well water at ambient temperature (approximately 26 C) was supplied through flow regulators at a constant rate of 1.9 l/min in each trough. Venturi drains were located at the ends of the troughs opposite the inflow. Supplemental aeration was provided through air stones. The water exchange rate in each trough was approximately 2.7 hrs. Dissolved oxygen was maintained in excess of 5.0 mg/l throughout the study with the exception of one 3-day period during the first experiment when pump failure occurred and the fish were subjected to static water until repairs were made. The fish were not fed during that period.

Three experimental rations were prepared by mixing DPW at 10, 20 and 30% of the total with No. 3 pellets of Master Mix trout feed (Central Soya and Subsidiaries, Ft. Wayne, Indiana). The DPW, composed of chicken manure and spilled chicken feed, was collected from under laying hens and was air dried prior to use. Unaltered trout feed was used as a control diet. Although the DPW was not incorporated into the trout feed, the fish did not discriminate against the DPW portion of any of the diets. Virtually all of the feed offered was consumed within minutes following presentation.

During the 1st experiment, 4 troughs received the control, 10, 20 and 30% DPW-supplemented diets, respectively, at 3% of the biomass of the trough daily for 10 wks. In the 2nd experiment, 2 additional troughs received the 20% DPW-supplemented diets at 1% and 5% of biomass daily for 8 wks. All fish were weighed at 2-wk intervals and the feeding rates were adjusted accordingly.

At the initiation of each experiment and at intervals of 2 wks thereafter, total biomass determinations were made on the fish in each trough. The average weight of the fish was determined by dividing the total weight by the number of fish present (survival was 100% in all troughs during both experiments). In order to keep handling to a minimum, individual weights were not obtained.

A proximate analysis was run on a sample of DPW utilizing standard analytical procedures (A.O.A.C., 1975). Amino acid analyses were run on a Beckman Model 120C amino acid analyzer.

RESULTS

The mean weights of *T. aurea* fed at 3% of body weight daily for 10 wks on control, 10, 20 and 30% DPW-supplemented diets are presented in Fig. 1. Fish receiving the control and 10% DPW-supplemented diets demonstrated similar growth, whereas after the first 4 wks of the experiment, slower growth was demonstrated by the other groups. Statistical comparison of the slopes of the regression lines presented in Fig. 1 (Snedecor and Cochran, 1967) indicated no significant differences among the 4 groups of fish ($P > 0.10$), although the trends followed a pattern of decreased growth with increased DPW-supplementation.

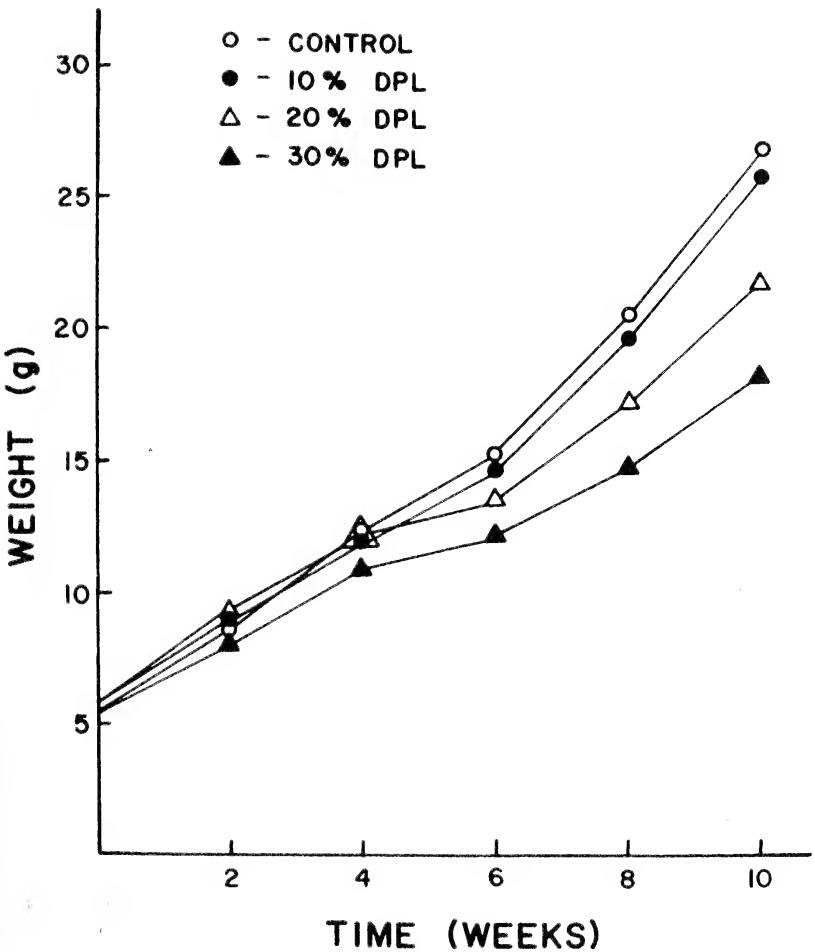


Figure 1. Mean body weight of *T. aurea* fed 3% of biomass daily for 10 wks on the control diet and diets supplemented with 10, 20 and 30% DPW.

Food conversions (dry weight of feed/wet weight of fish gain) were 1.02 and 1.06 for the control and 10% DPW-supplemented fish, respectively. The food conversion for fish supplemented with 20% DPW was 1.25, while that of the 30% DPW-supplemented fish was 1.40.

Growth curves of fish fed for 8 wks at 1% and 5% of body weight daily on the 20% DPW-supplemented ration are presented in Fig. 2. Included in that figure is the growth curve for the 20% DPW-supplemented fish fed at 3% of body weight daily during the 10-wk experiment. The slight suppression in growth between 4 and 6 wks for fish on the 3% feeding level corresponded with the 3-day pump failure, during which time the fish were not fed. A similar depression did not occur in the other 2 groups of fish (Fig. 2) since they were stocked subsequent to the pump failure.

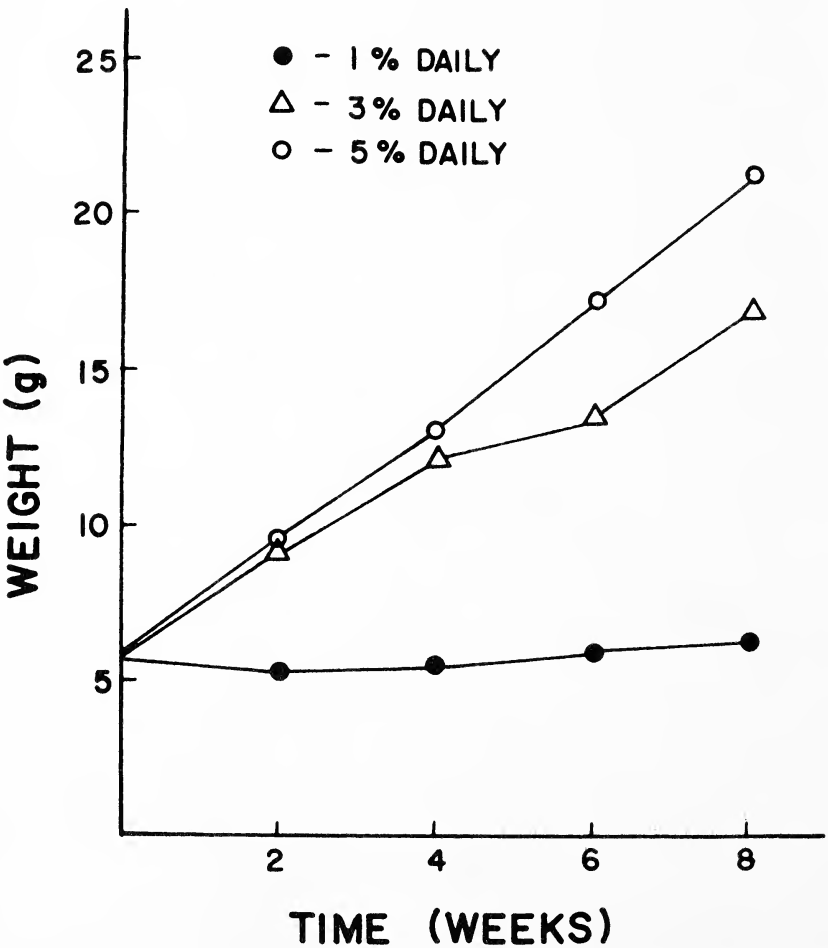


Figure 2. Mean body weight of *T. aurea* fed for 8 wks at 1, 3 and 5% of biomass daily on a diet supplemented with 20% DPW.

Proximate analysis of the DPW indicated that the material used in this study contained 26.2% protein, 12.5% fiber, 2.3% fat and 24.0% ash. Calcium and phosphorous levels were 7.7 and 1.5% of the total material, respectively. A partial amino acid analysis and the ammonia level found in the DPW are presented in Table 1.

TABLE 1

Amino Acid and Ammonia Content as a Dry Weight % of DPW

| Amino Acid | % of DPW |
|---|---|
| Alanine | 0.95 |
| Arginine | 0.33 |
| Aspartic Acid | 0.72 |
| Cystine | 0.32 |
| Glutamic Acid | 1.16 |
| Glycine | 1.90 |
| Histidine | 0.28 |
| Isoleucine | 0.49 |
| Leucine | 0.90 |
| Lysine | 0.72 |
| Methionine | 0.07 |
| Phenylalanine | 1.82 |
| Proline | 0.64 |
| Serine | 0.25 |
| Threonine | 0.32 |
| Tyrosine | 0.53 |
| Valine | 0.62 |
| Ammonia | 1.08 |

## DISCUSSION

The growth curves obtained from the diets fed at 3% of body weight daily demonstrated that increases in the level of dietary DPW resulted in slower growth compared with the control diet. Since these differences were not significant, higher levels of DPW-supplementation may have been possible without seriously retarding growth rate. Food conversions were outstanding for fish reared on the control and 10% DPW-supplemented diets. Even the poorest food conversion value obtained (1.40 from fish reared on the 30% DPW-supplemented diet) was excellent compared to other species of fish and to livestock.

With 20% DPW-supplementation, *T. aurea* can be maintained without growth if fed 1% of body weight daily (Fig. 2). A 5% daily feeding level appears to be excessive, since no increase in growth over feeding at 3% daily was apparent, although the higher feeding level might have resulted in faster growth at higher

temperatures than the average of approximately 26 C which occurred during this study. Further studies comparing controls at 1% and 5% daily feeding levels are required, but the lack of significant differences in growth with various levels of DPW-supplementation implies that the data in Fig. 2 would be representative for all diets used in the study.

DPW contains an average of about 10% true protein (Couch, 1974) in addition to other required nutrients. A potential problem associated with DPW is its high ammonia content, although elevated levels of ammonia were not observed during this study, nor in a study in which chicken droppings fell directly into a pond stocked with *T. aurea* (Stickney, *et al., In Press*). Similarly, when catfish were fed high levels of DPW in their feed, no ammonia toxicity was reported (Joe Lock, personal communication). *T. aurea* appear to have a high tolerance for ammonia. Stickney, *et al.*, (*In Press*) found that some survival occurred in a pond where ammonia levels in excess of 20 mg/l occurred consistently over a period of several weeks.

Among the essential amino acids present in DPW, methionine appeared to be the most limiting (Table 1). If DPW were utilized as a major source of protein in commercial *T. aurea* diets, additional methionine would probably be required. At present the amino acid requirements of *T. aurea* have not been determined, so further research is required before an assessment of the amino acid restrictions of DPW can be made.

This study gives a preliminary indication of the value of DPW in fish feed. Further studies to determine the highest levels of DPW supplementation possible without significant growth retardation are indicated. In addition, the DPW was not actually incorporated into the feed, even though the consumption rate and particle size of the control and DPW-supplemented feeds appeared to be similar. After the protein requirements of *T. aurea* are determined, the possibility of replacing significant portions of dietary protein with DPW should be investigated. Savings in feed costs without significantly reducing growth rate may be possible as long as the nutritional value of the feed is maintained.

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# DOG FLESH AS A POTENTIAL FOOD RESOURCE FOR CARNIVORES: AN EXPLORATORY STUDY

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ABSTRACT

The unowned dog population in the U.S. has become so large that it presents serious social and economic problems. Utilization of these dogs as a food resource for carnivores could eliminate the problems they cause while contributing to the total food supply. This paper is the first known effort to discuss this possible solution to the unowned dog problem and offer some exploratory research findings to support its thesis.

## INTRODUCTION

There are now between 10 and 20 million unowned dogs in the U.S. and their population growth is much more rapid than that of humans. At a meeting of the Texas Municipal League, it was noted that problems with unowned dogs represent the largest number of complaints to city mayors and are of major concern to public health authorities (Blackwell, 1976).

Problems from unowned dogs are numerous and serious. Among the problems are (1) livestock damage, (2) wildlife damage, (3) communication of several human diseases, some resulting in long-term illness and even blindness, (4) dog bites that may require painful treatment and cause physical disfigurement, (5) contamination that breeds flies, cockroaches and brown rats, (6) and of course, disposal of the dogs (Beck, 1974, 1975). The costs to the public of these problems exceed $500,000,000 annually (Blackwell, 1976).

Solutions to the unowned dog problem have been mainly 2, both of which add to the costs of the problem while producing minimal results. One is the disposal of the dogs by animal humane groups that "put them to sleep." Eighteen million dogs are put to sleep each year at a cost of $125,000,000 (Djerassi, *et al.*, 1973). The other solution is birth control among owned dogs including spaying, neutering and many birth control devices (Seager, 1976).

Converting this public liability to an asset should be given serious consideration. One possible solution may be to view the unowned dog as a food resource for other carnivores such as household pets and many zoo animals. If unowned dogs were slaughtered (instead of converted to ashes) and prepared as a food for

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household pets and zoo animals, 2 positive results would be (1) a reduction of surplus dogs, and (2) an increased availability of human foods by a reduction in the edibles now consumed by pets and the addition of the meats that are now eaten by feral dogs. This net increase in food available for human consumption should be of interest to those concerned with our hungry world.

The existence of aversion to feeding dog flesh to household pets is quite apparent. In spite of the fact that dog flesh is consumed by humans around the world, feeding that same flesh to treasured pets is likely to be unacceptable to many people. However, this aversion should not deter us from considering the feasibility of the idea. Desensitization therapy (Schaefer and Martin, 1975) is now in the realm of scientific status and probably could reduce the aversion over time through its application in the mass communications media. Also, aversion might be reduced by changing the name of the product just as we have done with fish roe (caviar) or calf thymus (sweetbread).

Recognizing the seriousness of the unowned dog problem, the investigators began an exploration into the feasibility of utilizing these dogs as a food resource. The slight possibility of solving the unowned dog problem while making a contribution to the world hunger problem were adequate incentives for the research effort.

## METHOD

The investigators assumed that food scientists had studied dogs as a food resource since dogs have been consumed for centuries by some humans around the world. Therefore, the first step taken in the exploration was an exhaustive search of the literature. The search was essentially unproductive. It produced some minor notes indicating some consumption of dog flesh by humans (Simoons, 1961). Two sources offered a 1-line presentation of estimated nutritional values of dog flesh with no supporting discussion (Spector, 1956; HEW, 1972). No empirical reports were found, however, about dog flesh as a food resource for carnivores.

The estimated nutritional values for dog flesh found in the literary research are presented in Table 1 along with values for lean beef and pork. A visual comparison among the 3 meats suggests that dog flesh generally possesses nutritional characteristics comparable to beef and pork. For example, protein and iron are slightly higher for dog, while calcium and niacin are lower. If one examines the nutritional contents of wet and moist commercial dog foods, he can see that the use of dog flesh in lieu of other meats such as beef and pork would not change the nutritional value of the feeds.

The consequence of the unproductive literary search combined with the encouraging interpretation of Table 1 was a mail survey conducted during the month of November, 1976, with 62 authorities in the field of nutrition. These experts were suggested by the Food and Nutrition Board of the National Academy of Sciences. Forty-four of the experts were affiliated with 36 universities in the

TABLE 1
Nutritional Composition of Lean Cattle, Pork and Dog*
(100 grams, edible raw portions)

| Component | Measure | Cattle | Pork | Dog |
|---|---|---|---|---|
| Water | % | 70–71.8[a] | 50.1–65[a] | 60.8–76[a] |
| Ash | g | 1.0 | 0.8 | 1.2 |
| Calcium | mg | 9–11[a] | 8 | 3.3–26[a] |
| Carbohydrate, Total | g | 0 | 0 | 0 |
| Chlorine | mg | 55[b] | NA | 50–75 |
| Cholesterol | % | 0.24[b] | 0.06[b] | 0.3 |
| Choline Phospholipid | % | 2.3[b] | NA | 2.9 |
| Fat | g | 7.2 | 35 | 23.5 |
| Fiber | g | 0 | 0 | 0 |
| Food Energy | cal | 150 | 376 | 274 |
| Iron | mg | 3.0 | 2.1 | 3.6 |
| Lipid | % | 10-14[b] | 8[b] | 14 |
| Magnesium | mg | 32[b] | 100[b] | 44 |
| Niacin | mg | 6.7 | 3.7 | 1.8 |
| Phospholipid | % | 3.7 | 3.1[b] | 5.8 |
| Phosphorus | mg | 171-210[a] | 151 | 185–219[a] |
| Potassium | mg | 489 | NA | 292–320[a] |
| Protein | % | 19.3[b] | 19[b] | 21 |
| Riboflavin | mg | 0.34 | 0.16 | 0.08 |
| Sodium | mg | 93 | NA | 74–131[a] |
| Thiamine | mg | 0.07 | 0.69 | 0.04 |

*Developed from Spector (1956) and HEW (1972)

[a]Combination of Both Sources
[b]Not Necessarily Lean

U.S., 2 in Canada, 1 in Taiwan, and 1 in Nigeria, 12 worked as nutritional specialists for U.S. food companies, and 6 were associates of hospitals in the U.S. They were asked to furnish any information or opinions regarding (1) the nutritional value of dog flesh, and (2) the feeding of dog flesh to household pets. Responses were received from 70% of the nutritionists.

The responses of the nutritionists were tabulated accordingly: (1) nutritionists who thought that dog flesh was nutritious, (2) nutritionists who felt dog flesh could be used as a pet food source, and (3) nutritionists who were concerned with the aversion factor. Also, an attempt was made to ascertain the degree of general knowledge about the nutrition of dog flesh held by the nutritionists.

Concurrently, a personal survey was conducted with a random sample of 121 pet owners during a 3-week period in November, 1976, in Bryan and College Station, Texas. The interviews were conducted by the authors and one student

in 3 supermarkets. The respondents were identified as a result of their selection of a cat or dog food from the supermarket shelf. The 2 objectives of this investigation were (1) to obtain an estimate of the amount of aversion held by pet owners to feeding canine flesh to their pets, and (2) to obtain estimates of aversion to other meats as pet foods for comparison. The respondents' aversive attitudes were categorized according to the respondents' age, gender, and social class.[1] The degree of aversion to dog flesh was expected to be high, but confirmation was desired. With a good estimate of the amount of aversion to dog flesh, particularly in comparison with other meats, some decision could be made about the possibility of reducing it through applications of desensitization therapy.

## FINDINGS

### *Survey of Nutritionists*

Approximately 2/3 of the responding nutritionists believed dog flesh to be nutritious. Most of the respondents compared the nutrition of dog flesh to that of pork, and 17.5% supported their belief with the HEW dog nutrition table mentioned above. Over 40% of the nutritionists felt that dog flesh could be used as an additional food source for household pets. A few of the respondents were, in fact, enthusiastic about the idea.

Half of the respondents noted the aversion factor. Some felt it might be circumvented; some felt it could not. A few voiced strong objections to the idea on the basis of being pet owners.

An interesting aspect of the survey was that 1/3 of the sample members said that they had no knowledge about the subject. Since mail surveys often are unanswered, particularly when the respondents have nothing to offer or are uninterested, this response was unexpected. It apparently demonstrates the professionalism of the sample members, and the interest in the topic by the group.

The mail survey appeared to produce 2 conflicting findings. It confirmed the unproductive literary search since no respondent referred to any research on the topic. However, the survey produced the impression that many nutritionists have knowledge and interest about dog flesh as a food resource. The interest was confirmed by the fact that 10 of the nutritionists offered to act as consultants on a funded project to study the topic in depth.

### *Survey of Pet Owners*

The main goal of surveying the pet owners was to obtain an estimate of their aversion to certain foods, including dog meat, that might be fed to *their* pets. Questions were asked about 3 groups of foods based on expected levels of aversion. The group of meats expected to elicit low aversion ($\leq$ 25% of n) consisted of pork, lamb and poultry. Fish and horsemeat, the second group, were expected to receive mild to strong aversion scores ($>$ 25%, $<$ 75% of n) from dog owners.

[1] Occupation provided a serviceable index for determining social class.

Similar scores for cat owners were expected for horsemeat, but fish was anticipated to produce a lower measure. The high aversion group of meats (> 75% of n) consisted of dog and armadillo. Table 2 presents a summary of the aversion to these meats according to respondents' age, sex and social class, and Table 3 summarizes the aversion by cat and dog ownership.

TABLE 2

Degree of Aversion to 7 Meats as Possible Ingredients in Dog and/or Cat Food by Age, Sex, and Social Class of Respondents

| Meat | Age | | | Sex | | Social Class | | |
|---|---|---|---|---|---|---|---|---|
| | 20-29 | 30-39 | 40 & Over | Male | Female | Lower | Lower Middle | Upper Middle |
| | (%) | | | (%) | | (%) | | |
| Poultry | 7 | 6 | 7 | 4 | 9 | 8 | 8 | 5 |
| Lamb | 5 | 20 | 23 | 17 | 16 | 24 | 24 | 11 |
| Pork | 32 | 40 | 44 | 17 | 30 | 48 | 44 | 35 |
| Fish | 22 | 17 | 16 | 19 | 20 | 20 | 18 | 20 |
| Horse | 27 | 23 | 21 | 15 | 29 | 24 | 26 | 21 |
| Armadillo | 22 | 37 | 40 | 29 | 36 | 45 | 44 | 26 |
| Dog | 42 | 57 | 67 | 46 | 64 | 68 | 70 | 47 |

TABLE 3

Degree of Aversion to 7 Meats as Possible Ingredients in Dog and/or Cat Food by Kind of Pet Owned

| Meat | Dog Owner | Cat Owner |
|---|---|---|
| | (%) | (%) |
| Poultry | 8 | 5 |
| Lamb | 18 | 14 |
| Pork | 39 | 40 |
| Fish | 27 | 7 |
| Horse | 21 | 28 |
| Armadillo | 35 | 30 |
| Dog | 54 | 61 |

The aversion to lamb and poultry was low regardless of age, sex or social class. However, there was much more aversion to pork (about 40% of sample) than to lamb (16%) and poultry (7%) for both cat and dog owners. The amount of aversion to fish and horse meat as pet foods was lower than expected. Aversion to fish for dogs was expected to be mild to strong. Actually, 73% of the sample said they would feed fish to dogs. The aversion to horsemeat was about the same for dog and cat owners (around 25%).

The expected very strong aversion to armadillo and dog meat was less than anticipated. Aversion to armadillo was only 26% among upper middle social class, and around 45% among lower and lower-middle pet owners. Aversion to armadillo increased with age, but only to a high of 40%. There was not much difference between male and female respondents' aversion to armadillo. Acceptance of armadillo for pet dogs was about the same as its acceptance for pet cats.

The major concern of the pet owner study, that is, the degree of aversion to dog meat as a pet food, proved to be a surprise. About 60% of people age 20-30 found dog meat acceptable, but only 39% of people over 30 accepted it. Over 1/2 of upper middle class respondents found dog meat acceptable for pet food, but only around 30% of lower and lower middle class pet owners accepted it. Aversion did not differ significantly between dog and cat owners. In general, aversion to dog meat as pet food was strong but not extreme.

## SUMMARY AND IMPLICATIONS

The research reported here was an initial exploratory study about the nutritional aspects of dog flesh and the amount of aversion among scientists and pet owners toward using it as a food for other carnivores. It is a small beginning, but offers more than presently exists in the literature.

A significant % of the nutritionists surveyed felt that dog flesh was a feasible food resource for house pets. In spite of the paucity of data about the nutritional value of dog flesh, a majority of those nutritionists in the study indicated it to be nutritious. Only 1/2 of the nutritionists were concerned with the aversion factor of consuming dog flesh. Since it is clearly stated in much literature how socially important the dog is in our society, the investigators intuitively anticipated the % to be much higher.

The survey results made it apparent that some nutritionists have knowledge and much interest in the food potential of dog flesh. However, the literature does not support this finding. Probably the aversion factor has caused the food scientists to not study dogs as a food source, or at least not to publish any results. As one respondent said, "I do not want to be associated with such an idea even though it may have merit."

Aversion to dog flesh as a pet food is not extreme among pet owners. In fact, the aversion to dog flesh among young people holding professional and managerial positions is only slightly higher than the aversion to pork as a pet food. Middle-aged women in lower middle class families had higher aversion to dog meat as pet food. In general, the research results reported here do not indicate that attempting to utilize dogs as a food resource for household pets is a futile task.

Besides the aversion factor, there is other opposition to utilizing dogs as an animal feed. Dog owners may feel that such a program is a threat to dog ownership. To the contrary, elimination of unowned dogs will remove "legal eyes" that are reviewing the dog population (Blackwell, 1976). Another probable element

of opposition to the proposed idea is pet food manufacturers. They may erroneously view it as a threat to their industry. However, at most what is being suggested is a change in some raw materials used by the pet food industry.

Finally, animal humane groups may be opposed to this suggested means for alleviating the unowned dog problem. Hopefully, these groups will see that the proposed program will terminate unwanted dogs as humanely as they would while having more effect on the unowned dog population. Hence, this proposed program is congruent with the goals of these 3 groups that might oppose it.

Using dog flesh as a food resource may be a viable alternative to solving the unowned dog problem. Its other merit is its contribution to the world hunger problem. It may be that experiments using unowned dogs as a food resource could be the beginning of a new industry comparable in nature to the beef or pork industry. An indication of the economic possibility is the fact that consumers spend $2 billion annually on commercial cat and dog foods.

Many serious questions must be answered, however, in addition to the nutritional and aversion aspects.

Some of these are concerned with

(1) Collecting and containing the dogs
(2) Deleting diseases in the dog carcasses
(3) Developing feeding and breeding procedures
(4) Developing slaughtering procedures
(5) Determining the final forms of the meat to be offered
(6) Determining the economic feasibility of the undertaking
(7) Dealing with local and national laws
(8) Convincing the pet food industry to use dog flesh as a food substitute or additive in lieu of other edibles.

In sum, the dearth of research reported on dog flesh as a food source is disappointing. The interest shown by the nutritionists surveyed on this subject, and the less-than-expected amount of aversion by pet owners, are encouraging. They suggest that basic interdisciplinary research should be undertaken to judge the value of using dog flesh as a food resource for carnivores. The social and economic pay-offs for such an effort could be substantial.

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# ESTIMATIONS OF THE RATIO OF INDUCED FISSION TO SPONTANEOUS FISSION IN URANIUM ORES[1]

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ABSTRACT

The mass spectrometric analysis of noble gases in uranium ores have been previously reported for geologically old uranium minerals. These data have been treated in a new method to determine the ratio of thermal neutron induced fission of $^{235}U$ to the spontaneous fission of $^{238}U$. The results indicate that xenon isotopes are more suitable for this method of treatment than the krypton isotopes. There is evidence to believe that in uranium minerals the basic modes of nuclear fission are the $^{235}U$ neutron-induced fission and the $^{238}U$ spontaneous fission. The results indicate that the ratio, R, for individual uranium mineral samples range from $\sim$0.5 to $\sim$0. Results are also consistent with previously reported radiochemical methods and the general geochemical composition of certain uranium minerals. Also, the self-sustaining nuclear reactor character of the Oklo Mine deposit is evident. The method is simple and provides a rapid means of determining this nuclear geochemical property of uranium minerals.

## INTRODUCTION

There have been no reports on the generalization of the nuclear fission phenomenon in uranium ores and minerals with respect to the spontaneous and induced fission processes. Several studies concerning the ratio of neutron induced fission of $^{235}U$ to the spontaneous fission of $^{238}U$ in uranium systems have been reported for natural uranium laboratory systems or a specific uranium ore (Kenna and Kuroda, 1960; Kenna and Attrep, 1966; Attrep and Sherwood, 1972; Raut and Attrep, 1975).

In discussing the nuclear geochemical parameter of the induced fission contribution in natural uranium systems, consideration must be given to the sources contributing to the total neutron population in the ore. These are neutrons arising primarily from the spontaneous fission of $^{238}U$, neutron-induced fission of $^{235}U$, and from ($\alpha$, n) reactions on low Z elements. Cosmic-ray neutron contributions have been shown to be negligible (Montgomery and Montgomery, 1939).

The isolation and identification of element 94, plutonium, from pitchblende, brought attention on the requirement that an additional neutron contribution must be present in addition to that supplied from the spontaneous fission of $^{238}U$.

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It was then speculated that $(\alpha, n)$ reactions on low Z elements might give the explanation (Peppard, *et al.*, 1951; Levine and Seaborg, 1951). Recently, in this laboratory (Attrep and Sherwood, 1972; Raut and Attrep, 1975) it has been shown that the ratio of induced fission to spontaneous fission in natural uranium can be increased by $(\alpha, n)$ reactions on beryllium as had been previously expected.

Some earlier mass spectrometric works (Macnamara and Thode, 1950; Fleming and Thode, 1953; Wetherill, 1953; Young and Thode, 1960) have investigated the fissiogenic isotopic composition of krypton and xenon in a number of uranium and thorium samples. The work of Young and Thode (1960) presented spontaneous fission spectra for both fissiogenic krypton and xenon isotopes. These mass spectrometric measurements of the noble gas fission products have enabled us to project the nuclear geochemical history of these minerals with respect to the induced fission of $^{235}U$.

## METHODS AND EQUATIONS

It will be assumed at this point that the total number of any fissiogenic isotope of krypton or xenon arises primarily from the spontaneous fission of $^{238}U$ and the thermal neutron-induced fission of $^{235}U$. With this basic assumption, the average ratio of the neutron-induced fission of $^{235}U$ to the spontaneous fission of $^{238}U$ can be estimated using the concentrations of these fissiogenic noble gas nuclides produced in uranium ores. The model presented here will allow a nuclear geochemical description of uranium ores considering the fission phenomena. In effect, this will reflect on the neutron population for the particular uranium mineral since the amount of neutron induced fission of $^{235}U$ is a direct consequence of the neutron fluence.

The number of atoms of a particular fissiogenic nuclide produced from the spontaneous fission process of $^{238}U$ can be expressed according to the following relationship:

$$N_S = N_{238}\,[\exp(\lambda_t^{238})T - 1]\frac{\lambda_{sf}^{238}}{\lambda_t^{238}}Y_s \qquad (1)$$

where $N_s$ is the number of atoms of the particular fissiogenic nuclide produced by the spontaneous fission of $^{238}U$; $N_{238}$ is the number of $^{238}U$ atoms present today in the sample; T is the fissiogenic retention time of the ore sample; $Y_s$ is the spontaneous fission yield for this fissiogenic nuclide; $\lambda_{sf}^{238}$ is the spontaneous fission decay constant for $^{238}U$; and $\lambda_t^{238}$ is the total decay constant for $^{238}U$.

In a similar manner, the number of atoms of the same fissiogenic nuclide produced by the thermal neutron induced fission of $^{235}U$ in the mineral can be expressed as follows:

$$N_i = N_{235}\,[\exp(\lambda_t^{235})T - 1]\frac{\Phi\sigma}{\lambda_t^{235}}Y_i \qquad (2)$$

where $N_i$ is the number of atoms of the fissiogenic nuclide produced from the thermal neutron induced fission of $^{235}U$ atoms initially present in the ore sample; $\Phi$ is the neutron fluence; $\sigma$ is the thermal neutron fission cross-section; $Y_i$ is the induced fission yield for this nuclide; and $\lambda_t^{235}$ is the sum of all the decay constant process for $^{235}U$.

The overall ratio, R, for the thermal neutron-induced fission of $^{235}U$ to the spontaneous fission of $^{238}U$ experienced in the uranium mineral is expressed as the total number of induced fission events in relation to the total number of spontaneous fission events for the same time period, T, the retention time of the ore. This is expressed as follows:

$$R = \frac{N_{235}[\exp(\lambda_t^{235})T - 1]\frac{\Phi\sigma}{\lambda_t^{235}}}{N_{238}[\exp(\lambda_t^{238})T - 1]\frac{\lambda_{sf}^{238}}{\lambda_t^{238}}} \tag{3}$$

For one fissiogenic nuclide, the total concentration is expressed as $N_t'$. This will represent the concentration of that nuclide in the uranium ore. This concentration is assumed to be fissiogenic and is assumed to be comprised of 2 components: spontaneous fission produced, $N_s'$, and induced fission produced, $N_i'$. Thus, $N_t' = N_S' + N_i'$ and the total concentration of a specific fissiogenic nuclide in a uranium ore is

$$N_t' = N_{238}[\exp(\lambda_t^{238})T - 1]\frac{\lambda_{sf}^{238}}{\lambda_t^{238}}Y_s' + N_{235}[\exp(\lambda_t^{235})T - 1]\frac{\Phi\sigma}{\lambda_t^{235}}Y_i' \tag{4}$$

By combining equations (3) and (4) and making appropriate rearrangements, $N_t'$ is expressed as a function of the value R as shown below:

$$N_t' = N_{238}[\exp(\lambda_t^{238})T - 1]\frac{\lambda_{sf}^{238}}{\lambda_t^{238}}(Y_s' + RY_i'). \tag{5}$$

If the absolute concentration value of the particular fissiogenic nuclide is known, along with the other parameters including the retention time for the ore, the ratio of induced fission to spontaneous fission can be obtained. However, for the most part, these values are unattainable, and some of those which probably could be estimated may be unreliable.

Upon inspection, it is noted that a similar expression can be written for another fissiogenic nuclide in the same ore:

$$N_t'' = N_{238}[\exp(\lambda_t^{238})T - 1]\frac{\lambda_{sf}^{238}}{\lambda_t^{238}}(Y_s'' + RY_i''). \tag{6}$$

If equation (5) is divided by equation (6), the ratio of the total number of atoms of one fissiogenic isotope in relation to another can be expressed as follows in

which this relationship is a function of R the fission yields of the isotopes in question, and the ratio of 2 fissiogenic nuclides. This is expressed as follows:

$$\frac{N'_t}{N''_t} = \frac{(Y'_s + RY'_i)}{(Y''_s + RY''_i)} \tag{7}$$

As shown in equation (7), the observed ratio of fissiogenic products is not a function of T, but is a function of single variable, R, the average ratio of thermal neutron-induced fission of $^{235}U$ to the spontaneous fission of $^{238}U$. The fissiogenic nuclides used in this study are the krypton and xenon isotopes. The fission yields for the spontaneous fission process of $^{238}U$ and the thermal neutron-induced fission process of $^{235}U$ are shown in Table 1. The yields for $^{129}Xe$ have been omitted because the spontaneous fission yield is set at a lower limit at $<0.012\%$ (Wetherill, 1953). The spontaneous fission yields used in this study are those of Young and Thode (1960).

TABLE 1

Percent fission yields of xenon and krypton isotopes form $^{238}U$ spontaneous fission and $^{235}U$ thermal neutron induced fission.

| Mass Number | $^{238}U$ Spontaneous Fission[a] % Yield | $^{235}U$ Neutron Induced Fission[b] % Yield |
|---|---|---|
| Krypton Isotope | | |
| 83 | 0.0327 ± 0.0028 | 0.561 ± 0.012 |
| 84 | 0.1220 ± 0.0121 | 1.032 ± 0.022 |
| 86 | 0.9510 ± 0.0570 | 2.036 ± 0.019 |
| Xenon Isotope | | |
| 131 | 0.524 ± 0.031 | 2.92 ± 0.02 |
| 132 | 3.630 ± 0.220 | 4.35 ± 0.04 |
| 134 | 5.140 ± 0.310 | 7.93 ± 0.24 |
| 136 | 6.300 ± 0.380 | 6.39 ± 0.11 |

[a]From Young and Thode, (1960).

[b]The % yields presented are an average of 5 reported values, as presented in Hyde (1964); the errors associated are "standard deviations."

Using equation (7) and the fission yields given in Table 1, the relationship between R, the ratio of induced to spontaneous fission, and the fissiogenic noble gas pairs are produced. Variation of R with the ratios of the total number of fissiogenic isotopes of krypton pairs are shown in Fig. 1 for the 3 combinations: $^{86}Kr/^{83}Kr$, $^{86}Kr/^{84}Kr$ and $^{84}Kr/^{83}Kr$. In a similar manner, Fig. 2 shows the ratio of the total number of atoms of one xenon isotope to that of another versus R. From inspection of Fig. 1 it appears that a small variation of R, the ratio of induced to spontaneous fission, is more readily reflected in the relatively large

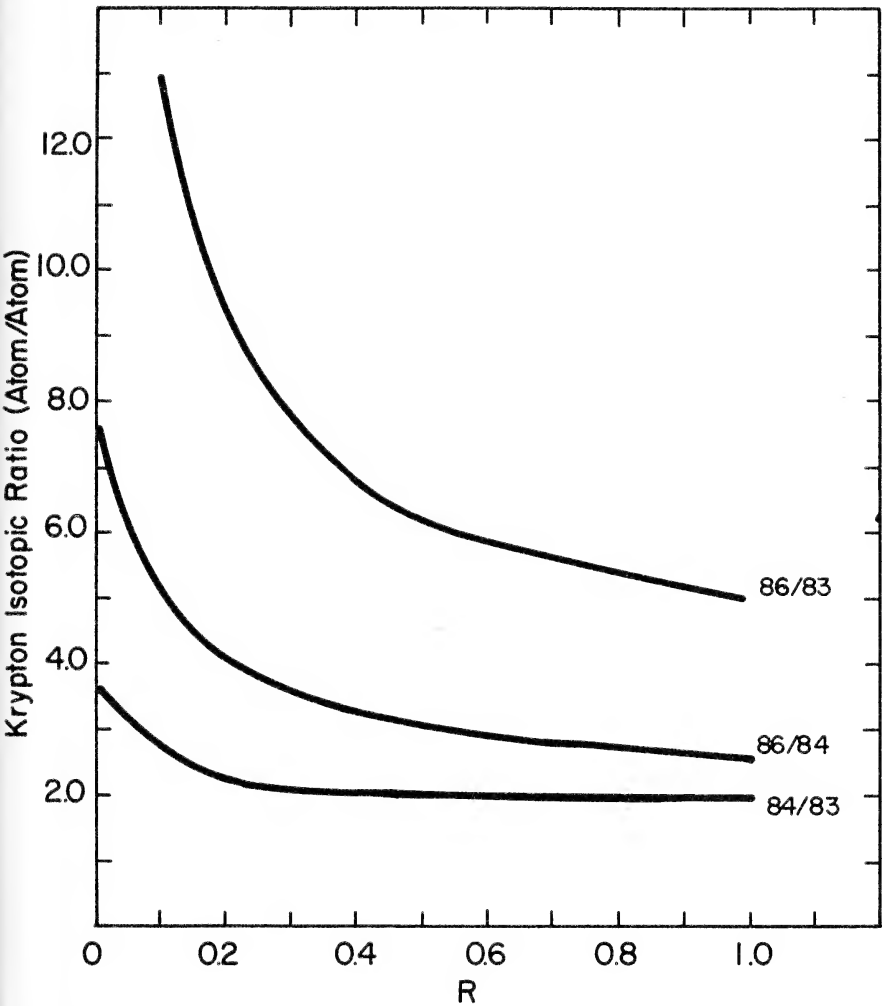


Figure 1. The average ratio of induced fission of $^{235}U$ to spontaneous fission of $^{238}U$ as a function of ratio of krypton isotopic ratios.

change of the 86/84 and 86/83 krypton ratios as compared to that for the krypton pair 84/83. Also, from Fig. 2 the same conclusion can be drawn from the xenon isotopes. The xenon isotope pairs of 134/131, 136/131 and 132/131 exhibit the most useable relationships.

## RESULTS AND DISCUSSION

Treatment of the reported mass spectrometric analyzed noble gas nuclides in a number of uranium minerals using the relationship devised (equation 7) yields

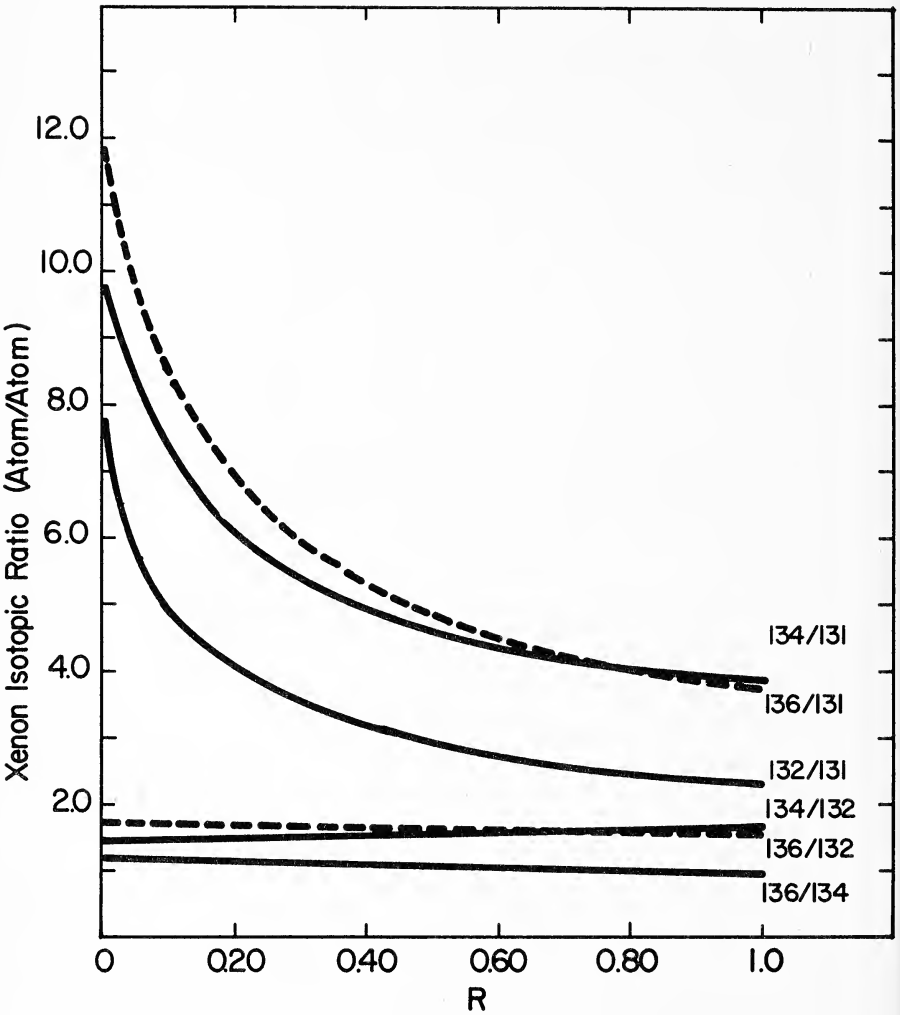


Figure 2. The average ratio of induced fission of $^{235}U$ to spontaneous fission of $^{238}U$ as a function of ratio of xenon isotopic ratios.

values of R. The evaluated R values from the 3 krypton isotope pairs and from the 3 xenon isotope pairs are presented in Table 2. The % $U_3O_8$ and the age of the ores are those given by the authors from which the noble gas values were taken. The type of uranium sample analyzed is predominately pitchblende; however, there are a few uraninite samples. The name and location of the uranium minerals are also those reported in the original papers.

The R values derived from the noble gas isotopic ratios exhibit some features which are of importance for further discussion of the nuclear fission phenomenon in uranium minerals. There is consistency of the value of R for the 3 sets of xenon

TABLE 2

Derived values for the ratio of thermal neutron induced fission of $^{235}U$ to the spontaneous fission of $^{238}U$ in uranium ore samples.

| Sample | $\%U_3O_8$ | Age (million years) | R for Xenon Isotopes $\frac{132}{131}$ | $\frac{134}{131}$ | $\frac{136}{131}$ | Average | R for Krypton Isotopes $\frac{86}{84}$ | $\frac{86}{83}$ | $\frac{84}{83}$ | Average | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pitchblende, Katanga, Belgian Congo | 65.21 | 640 | 0.42 | 0.42 | 0.40 | 0.41 | 0.42 | 0.77 | >1 | 0.73 | Fleming and Thode (1953) |
| Pitchblende, Belgian Congo | - | 650 | 0.42 | 0.42 | 0.44 | 0.43 | 0.44 | 0.32 | 0.17 | 0.31 | Wetherill (1953) |
| Pitchblende, Belgian Congo | 66.5 | 635 | 0.46 | 0.48 | 0.48 | 0.47 | 0.39 | 0.37 | 0.30 | 0.35 | Young and Thode (1960) |
| Pitchblende, Eagle Mine, Beaverlodge | 45.5 | 1630 | 0.34 | 0.33 | 0.34 | 0.34 | -- | -- | -- | -- | Fleming and Thode (1953) |
| Pitchblende, Eagle Mine | 53.4 | 1700 | 0.26 | 0.25 | 0.26 | 0.26 | 0.26 | 0.22 | 0.16 | 0.21 | Young and Thode (1960) |
| Pitchblende, Ace Mine, Beaverlodge | 16.96 | 1600 | 0.27 | 0.28 | 0.28 | 0.28 | -- | -- | -- | -- | Fleming and Thode (1953) |
| Pitchblende, Beaverlodge Lake | 48.6 | 785 | 0.08 | 0.09 | 0.09 | 0.09 | 0.02 | 0.08 | 0.19 | 0.10 | Young and Thode (1960) |
| Pitchblende, Nesbitt Labine, Beaverlodge | 27.55 | - | 0.13 | 0.15 | 0.13 | 0.14 | - | -- | -- | -- | Fleming and Thode (1953) |
| Pitchblende, Lake Athabaska | 13.99 | 1690 | 0.12 | 0.12 | 0.12 | 0.12 | - | - - | - | - - | Fleming and Thode (1953) |
| Pitchblende, Great Bear Lake | 36.46 | 1370 | 0.11 | 0.13 | 0.12 | 0.12 | 0.33 | 0.08 | <0 | 0.14 | Fleming and Thode (1953) |
| Pitchblende, Great Bear Lake | | | 0.12 | 0.13 | 0.12 | 0.12 | 0.30 | 0.09 | 0.0 | 0.13 | Macnamara and Thode (1950) |
| Pitchblende, Great Bear Lake | 51.1 | 1400 | 0.23 | 0.24 | 0.24 | 0.24 | 0.19 | 0.19 | 0.19 | 0.19 | Young and Thode (1960) |
| Uraninite, Jahala Lake | 35.8 | 1740 | 0.04 | 0.04 | 0.04 | 0.04 | 0.09 | 0.05 | 0.04 | 0.06 | Young and Thode (1960) |
| Uraninite, Cardiff Township, Ontario | 71.0 | - | 0.03 | 0.03 | 0.03 | 0.03 | | | - - | - | Fleming and Thode (1953) |
| Uraninite, Cinch Lake | 34.2 | 1120 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.05 | 0.08 | 0.06 | Young and Thode (1960) |
| Pitchblende, Rabbit Lake | 5.3 | 1200 | 0.16 | 0.16 | 0.16 | 0.16 | 0.0 | 0.08 | >1 | 0.36 | Drozd, *et al.*, (1974) |
| Pitchblende, Rabbit Lake | 3.2 | 1200 | 0.04 | 0.04 | 0.04 | 0.04 | 0.0 | 0.03 | 0.13 | 0.05 | Drozd, *et al.*, (1974) |
| Pitchblende, Rabbit Lake | 10.3 | 1200 | 0.05 | 0.05 | 0.05 | 0.05 | 0.0 | <0 | <0 | 0.0 | Drozd, *et al.*, (1974) |
| Pitchblende, Oklo Mine | 7.6 | 1700 | >1 | >1 | >1 | >1 | >1 | >1 | >1 | >1 | Drozd, *et al.*, (1974) |
| Pitchblende, Oklo Mine | 17.5 | 1700 | >1 | >1 | >1 | >1 | >1 | >1 | >1 | >1 | Baudin, *et al.*, (1972) |

isotopes for all samples. This is not the case for those values of R arising from the krypton isotope ratios. In general, however, there is poor agreement between those R values from the krypton isotopes and those from the xenon isotopes. It should be noted that the experimental data used for the krypton isotopes have been reported with errors in some cases up to 100%. In view of the experimental error involved our discussion concerning the ratio, R, will be drawn primarily from the xenon isotopic values.

The 3 Belgian Congo pitchblende samples show similar R values that are very high in relation to the other samples. For these 3 samples the values of R are 0.47, 0.43, and 0.41. It is not known if this is one single sample analyzed by the 3 separate laboratories. Aside from the Oklo Mine sample which had undergone a self-sustaining nuclear fission reaction, these samples have the highest contribution of thermal neutron induced fission. From the determination of $^{36}Cl(t_{1/2} = 3 \times 10^5$ years) from African (Belgian Congo) pitchblende and Canadian (Great Bear Lake) pitchblende, Kenna and Kuroda (1960) reported R values of 0.33 and 0.19, respectively. The African pitchblende samples have somewhat higher values of R than that reported for the $^{36}Cl$ work. However, the radiochlorine value of R = 0.19 is in good agreement with the Great Bear Lake samples reported in Table 2, i.e., R = 0.12, 0.12, and 0.24. Two considerations must be made, however. First, because the samples for mass spectrometric analysis and radiochemical analysis are probably not identical there may be major chemical composition factors bringing about these differences if they are significant. Secondly, the radiochlorine work represents the ratio of induced fission to spontaneous fission over the past 3 million years whereas the mass spectrometric xenon isotope value for R includes essentially the lifetime of the mineral, up to over 600 million years. The ratio of induced to spontaneous fission measured by stable isotopes is an average value over the retention age of the sample. Due to the shorter half-life of $^{235}U$ with respect to $^{238}U$, the $^{235}U$ concentration would be enriched in the geological past and the ratio of induced fission to spontaneous fission measured in this manner would be different from that determined by a radionuclide.

Non-homogeniety of either chemical or physical properties of the mineral can yield variations in the value of R. For example, the variation of R within a particular pitchblende deposit is well pronounced in the Beaverlodge pitchblende samples. R ranges from 0.09 to 0.34. Significant increases in the contribution of thermal neutron induced fission of $^{235}U$ is probably due to the presence of low Z elements like beryllium which are capable of increasing the total neutron flux by ($\alpha$, n) reactions (Attrep and Sherwood, 1972; Raut and Attrep, 1975). Variations in chemical composition of elements capable of undergoing ($\alpha$, n) reactions would account for such a variation of induced fission contribution in the samples.

The presence of elements such as the lanthanides may decrease this neutron flux due to their characteristically high neutron absorption cross-sections. This effect is observed in the 3 uraninite samples. Uraninites usually contain lanthanides and would exhibit low values of induced fission. For these samples, R = 0.03,

0.03, and 0.04, respectively. These results are consistent with those obtained by Young and Thode (1960) using complete spectral analysis. Slight variations of the concentrations of either group of elements from location to location will definitely alter the thermal neutron-induced fission factor.

Figure 3 is a plot for the xenon isotopes 134/131 versus 136/131. Similarily in Fig. 4 a xenon isotopic composition graph for 134/132 versus 136/132 is shown. In each figure the points which represent the pure spontaneous fission of

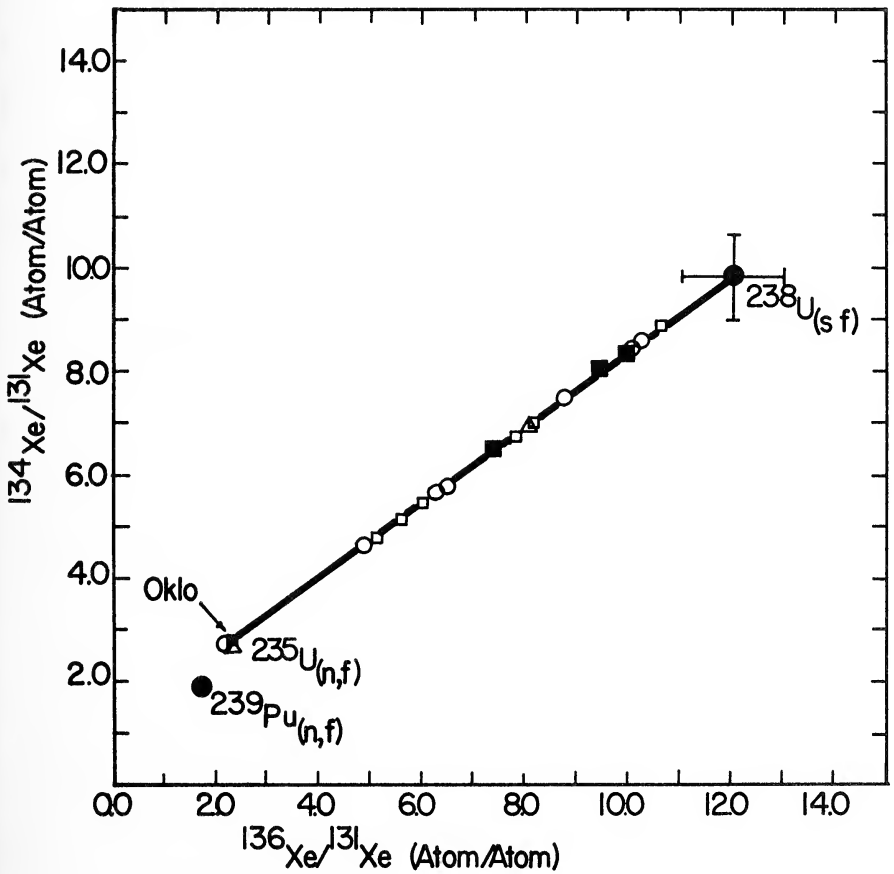


Figure 3. $^{134}Xe/^{131}Xe$ versus $^{136}Xe/^{131}Xe$ plot for uranium minerals.

$^{238}U$ and thermal neutron-induced fission of $^{235}U$ are designated. We have also included the point representing $^{239}Pu$ neutron-induced fission (Hyde, 1964). The individual points are not identified except for the Oklo mine samples.

In Fig. 3, it is quite evident that all points lie on a line between the values of pure spontaneous fission of $^{238}U$ and the thermal neutron-induced fission of $^{235}U$.

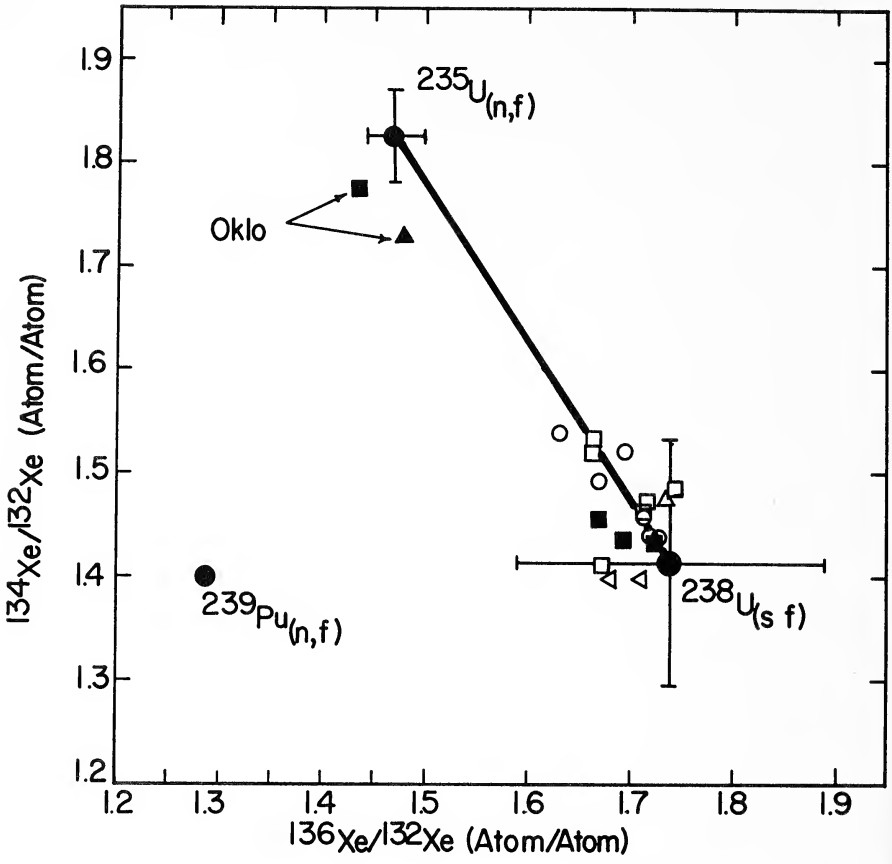


Figure 4. $^{134}Xe/^{132}Xe$ versus $^{136}Xe/^{132}Xe$ plot for uranium minerals.

This trend strengthens our original assumption that in uranium ores there are basically only the 2 types of fission processes previously stated. Also, if the graphical method of analysis of Podosek (1970) is employed for Fig. 3 in order to establish the amounts of the individual xenon isotopes originating from the thermal neutron-induced fission of $^{235}U$ and the spontaneous fission of $^{238}U$, the same R values are obtained for each sample which are derived by the first proposed method.

The Oklo Mine sample shows the induced fission component to be extremely dominant. From Table 2 the R value is >1. This agrees with the evidence for a naturally self-sustaining chain reaction taking place in this unique uranium deposit (Baudin, *et al.*, 1972). (An excellent review of this natural nuclear reactor has been recently published by Cowan (1976).) The chain reactor aspect of the Oklo Mine is clearly indicated from our proposed treatment of the mass spectrometric data of the xenon isotopic composition and the isochronic treatment of the data of Fig. 3 and 4.

In Fig. 4 using the xenon isotopic pairs 134/132 versus 136/132, the same trend is expressed as in Fig. 3. However, this trend is not as neat. The purpose of plotting the $^{239}Pu$ value, especially in Fig. 3, is to indicate the possibility of this mode of fission in the self-sustaining chain reactor ore, the Oklo Mine. Drozd, *et al.,* (1974) and Baudin, *et al.,* (1972) have both indicated that this type of fission occurred in this uranium deposit. Although it is not evident in Fig. 3 because the $^{239}Pu(n, f)$ point lies close to the $^{235}U(n, f)$ point, it does become somewhat convincing in Fig. 4 that the $^{239}Pu$ neutron induced fission process occurred in this deposit.

## CONCLUSION

This treatment of the noble gas mass spectrometric analysis of uranium ores using our initial approach indicates that our basic approach to the nuclear geochemical phenomena of fission is correct. The use of xenon isotopes is more reliable than those data of the krypton isotopes. The following are concluded from this study: (a) there are predominately 2 modes of fission in normal uranium minerals: the spontaneous fission of $^{238}U$ and the therman neutron-induced fission of $^{235}U$; (b) the thermal neutron-induced fission of $^{235}U$ contributes up to about 30% of the total fission events in the ore; (c) the Oklo Mine samples are the only uranium mineral samples which clearly demonstrate a predominance of $^{235}U$ thermal neutron induced fission giving support to the idea that it was a natural nuclear chain reactor; (d) there appears to be no correlation between age, uranium content and the ratio of induced to spontaneous fission in the uranium samples.

## ACKNOWLEDGEMENT

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# MASS SPECTROMETRY STUDIES OF NORCAMPHOR AND NORBORNYL ACETATE

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ABSTRACT

Six norcamphor ( 1 ) derivatives variously specifically deuterated were synthesized and studied by mass spectrometry to ascertain fragmentation patterns. Extensive deuterium scrambling occurs, attesting to facile hydride migrations, but the fragmentation pattern of norcamphor closely parallels that of the trimethyl analog camphor. A brief look at 2-norbornyl acetate ( 3 ) and a deuterio derivative indicates less scrambling occurs in this system than in 1 and that the ketone function actually promotes hydride migrations.

## INTRODUCTION

The mass spectra of acyclic and monocyclic ketones have been studied extensively and commonly involve an initial α-cleavage with subsequent hydride shifts and rearrangements (Budzikiewicz, *et al.,* 1964). Studies for compact polycyclic ketones are fewer, but an initial α-cleavage is indicated (Weinberg and Djerassi, 1966; Sydow, 1964; Dimmel and Wolinsky, 1967). In general, polycyclic compounds are capable of more complicated and less predictable reactions, and previous studies of bicyclo[2.2.1]heptanones demonstrate this possible complexity in such systems (Weinberg and Djerassi, 1966; Sydow, 1964; Dimmel and Wolinsky, 1967). However, these studies have dealt with methyl-substituted systems, and it has not been clear to what extent these methyl substituents directed the rearrangement process. Accordingly, in the present study, unsubstituted norcamphor ( 1 ) was studied. In this study, various deuterated norcamphor derivatives ( 1b-f ) were synthesized and subjected to mass spectral analysis. Undeuterated norcamphor itself ( 1a ) has been briefly analyzed by mass spectrometry (Goto, *et al.,* 1966), but no conclusions were reached other than it appeared that a $CH_3CO$ radical was lost.

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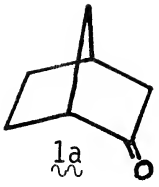
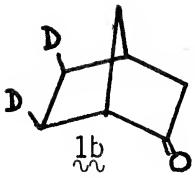



1a

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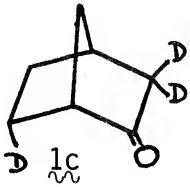


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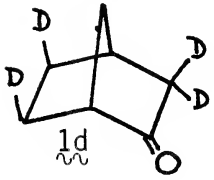
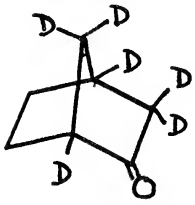
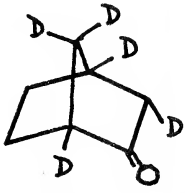


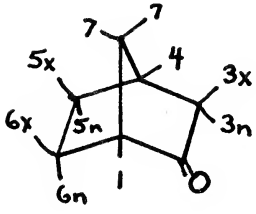

1d




1e



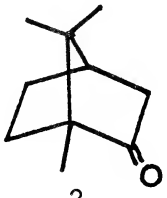

1f




1

## RESULTS AND INTERPRETATION

The mass spectral peaks for 1a-f are given graphically in Fig. 1. Mechanisms are given below to account for these patterns and are then compared with those proposed for camphor 2 (Weinberg and Djerassi, 1966; Dimmel and Wolinsky, 1967).

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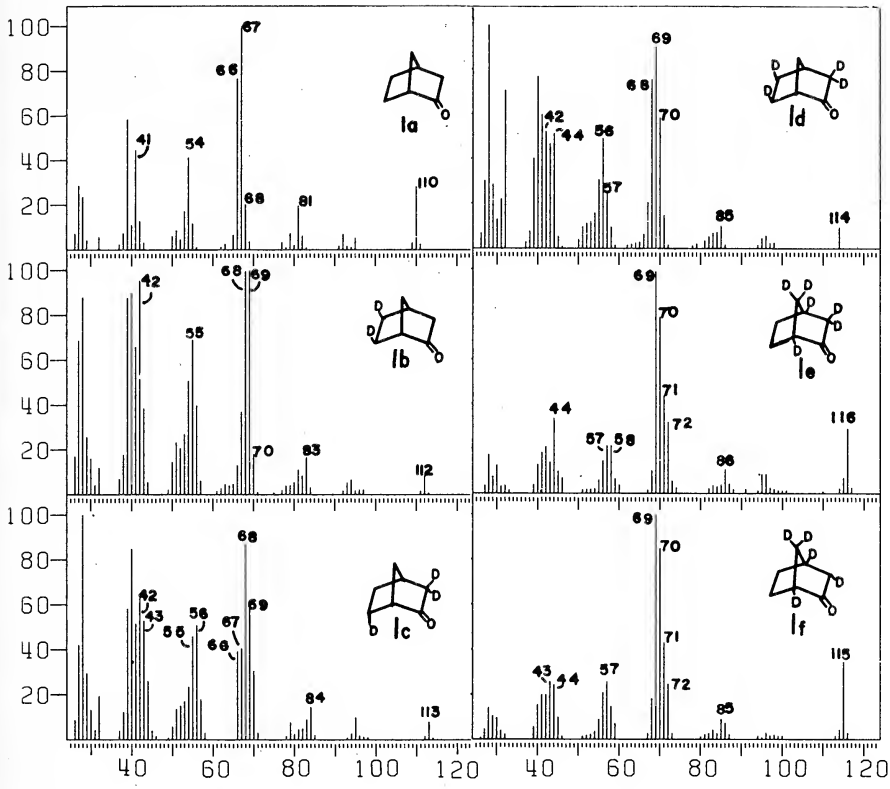
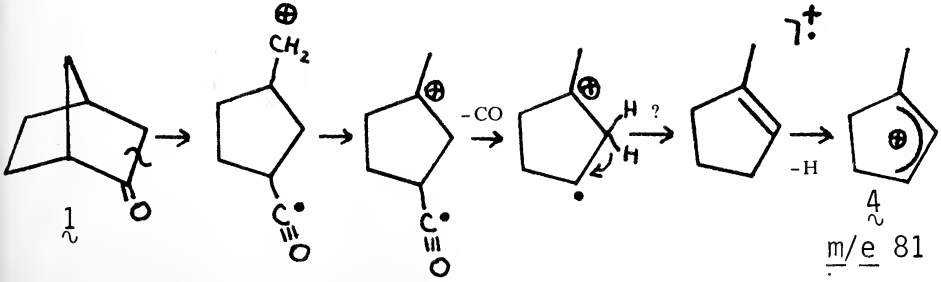
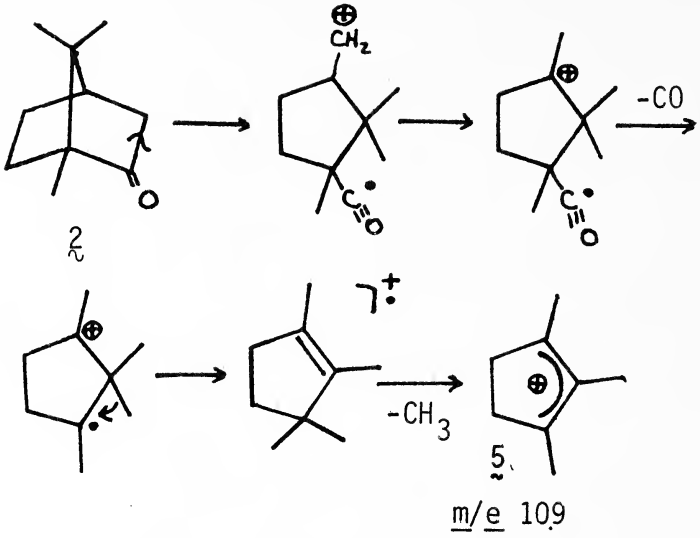


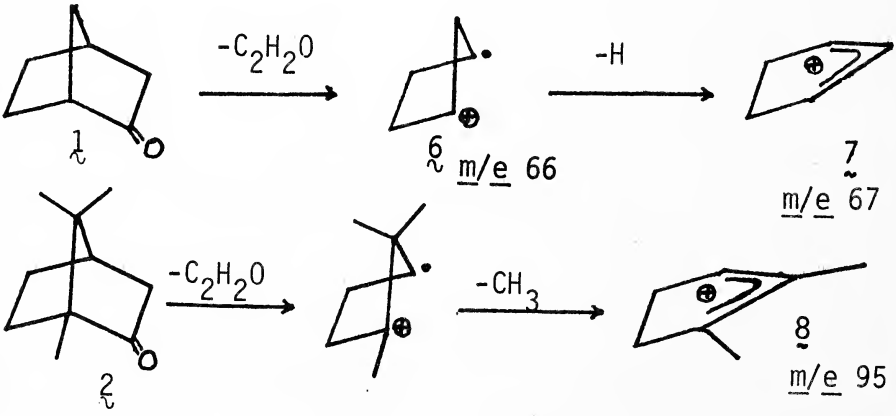

Figure 1. Mass spectra (relative abundance *vs. m/e*) for norcamphor ( 1a ) and deuterio derivatives 1b-f. The origins of peaks specifically identified are discussed in the text.

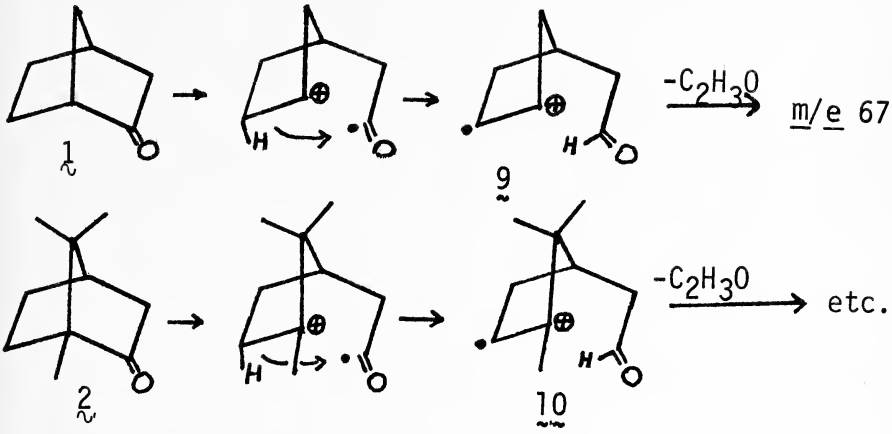
In the first spectral region (*m/e* 81), norcamphor loses CO (but not ethylene because the deuteriums are retained in 1b ) and a hydrogen to form the methylcyclopentenyl cation 4; this mechanism is mirrored closely in the fragmentation patterns for camphor ( 2), which gives as a base peak *m/e* 95, proposed (Weinberg and Djerassi, 1966; Dimmel and Wolinsky, 1967) as 5. The mass spectral patterns for 1a-f indicate that C-7 hydrogen is lost, consistent with the proposed mechanism for camphor (Weinberg and Djerassi, 1966; Dimmel and Wolinsky, 1967) that a C-7 methyl group is lost.






In the next spectral region (*m/e* 66, 67) norcamphor is proposed to lose a ketene to give 6, and then a hydride to give 7. Similarly, camphor loses ketene and a methyl group to give 8, *m/e* 95. For camphor, it was shown (Dimmel and Wolinsky, 1967) that the ketene arises from the C2-C3 linkage, and likewise the patterns for 1a-f indicate the same type of loss for norcamphor (for example, the 2 deuteriums are retained in 1b). A remarkable similarity between camphor (Weinberg and Djerassi, 1966, Dimmel and Wolinsky, 1967) and norcamphor is the abstraction of a hydride by the C1-C2 α-cleavage intermediate in a 6,2-shift process to give 9 and 10 respectively (Bartlett, 1965). The necessity for proposing such a 6,2-shift for norcamphor is prompted because it appears that sometimes C7 hydrogens are retained (see 1e,f).



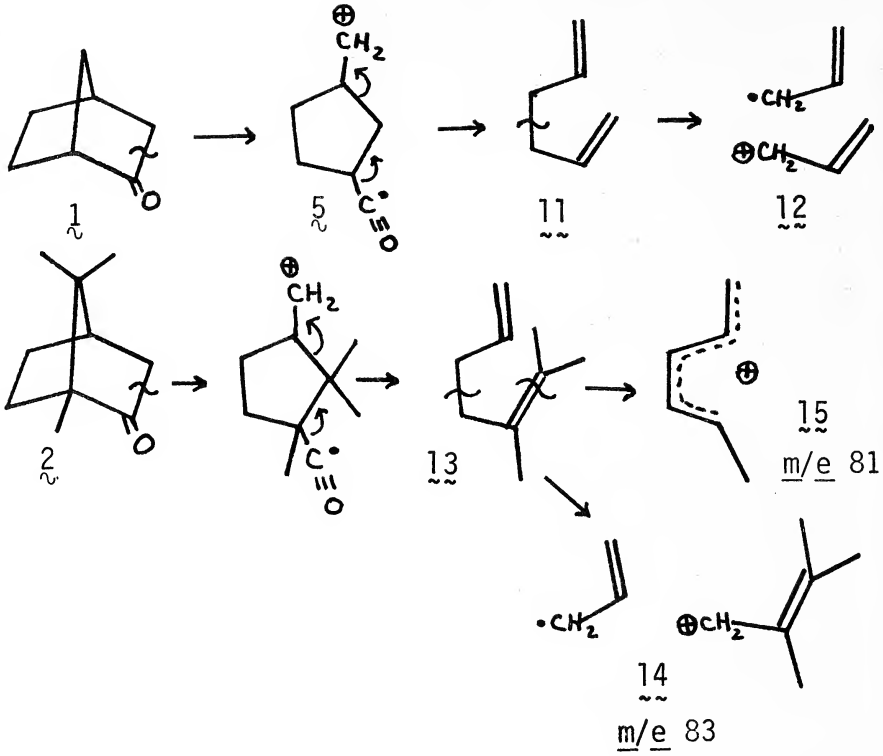



The next spectral region (*m/e* 54) corresponds to a $C_4H_6$ fragment. For camphor a precisely corresponding fragment does not exist, but there does exist a peak at *m/e* 81 that corresponds to the retention of two methyl groups but the loss of an extra hydrogen. Thus, it is not clear whether the correlation between the fragmentation patterns of camphor and norcamphor extends into this region. The spectral patterns for 1a-f indicate that the origin of this *m/e* 54 peak is the C3, C4, C5, C7 assemblage.

Finally, the origin of the *m/e* 41 peak for norcamphor is proposed as below. For camphor (2) this same process was proposed to give the fragment 13, which through a series of hydride transfers and loss of $C_3H_7$ gave 15, *m/e* 81 (Weinberg and Djerassi, 1966; Dimmel and Wolinsky, 1967). In norcamphor, however, no such $C_3H_7$ fragment could be lost; instead, a more direct sequence would be to symmetrically cleave 11 as shown. If this were correct, then an analogous sequence should be observed for camphor—13 would cleave to give 14, with the positive charge preferentially on the lower half, *m/e* 83. This peak is indeed observed for camphor (Weinberg and Djerassi, 1966; Dimmel and Wolinsky, 1967; Sydow, 1964) and precisely this mechanism has been proposed to account for its existence.

## COMPARISON OF NORCAMPHOR WITH NORBORNYL ACETATE

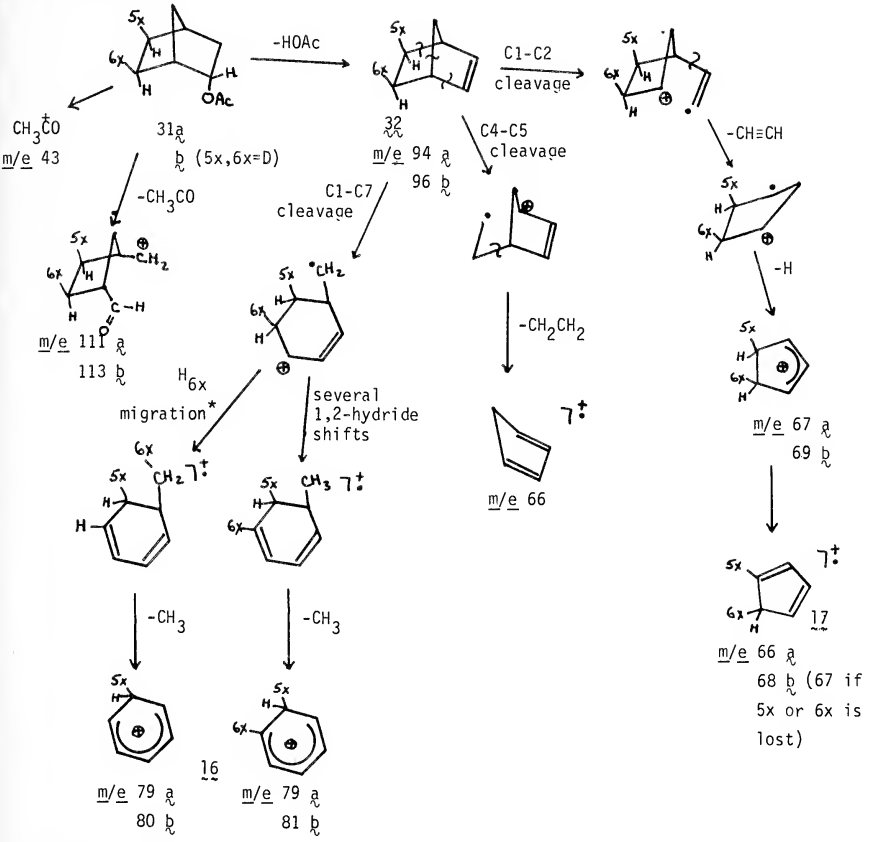
The norcamphor system 1 appears to exemplify that relatively simple terpenes can undergo quite complicated fragmentations in their compact framework in the absence of powerful directing groups. In fact, the carbonyl group seems to complicate matters. To demonstrate this, the mass spectral data of *endo*-2-norbornyl acetate 3a and its *exo,exo*-5,6-dideuterio derivative 3b (Figure 2) can be interpreted rather extensively, as shown in the scheme below. The only indication of scrambling is apparent at *m/e* 80 for 16; if 5x or 6x migrates and is lost, then overall only one deuterium will be lost.




The fragmentation pattern given in the scheme on the following page is consistent with mechanisms proposed for 1- and 2-methylnorbornenes (Reed, 1963) in that the retro Diels-Alder reaction is not the principal method of fragmentation in norbornenes (a retro Diels-Alder reaction for 3 would give *m/e* 66 directly with no fragments *m/e* 67-69). Apparently norbornene derivatives need a better dienophile leaving group; hence, norbornenyl acetate gives virtually quantitative retro Diels-Alder reaction (Cristol, *et al.*, 1966). A retro Diels-Alder mechanism has been proposed to be general for norbornenes and benzonorbornenes, (Goto, *et al.*, 1966) but we suggest that the loss of two carbon fragments from these compounds is a stepwise process, as indicated in the scheme.

## CONCLUSIONS

Although fragmentation patterns for small, compact molecules may be complex, and although the ketonic functionality (at least in the present study) does not promote clean fragmentation patterns, mass spectral analysis for norcamphor




*Alternatively, $H_{5x}$ migration and subsequent hydride shift will give the same overall effect.

may be done by means of various deuterated derivatives. The fragmentation patterns that appear to emerge from this analysis are quite analogous to those for the trimethyl substituted analog camphor. Therefore, this study indicates that fragmentation patterns for the bicyclo[2.2.1]heptan-2-one system remains relatively the same with or without methyl substitution and attests to the mechanistic integrity of the carbon skeleton itself in mass spectral fragmentation patterns.

## EXPERIMENTAL

*Norcamphor Derivatives 1a-f*

This synthesis of these derivatives has been previously described (Marshall and Walter, 1974) and a very close proton nmr study (Marshall and Walter, 1974) of 1b-f proves the deuteriums are cleanly substituted with negligible admixture of isomers or compounds with lesser amounts of deuterium. Mass spectral analysis gave the following compositions: 1b, 86.6% $d_2$, 8.9% $d_1$, 4.4% $d_0$; 1c, 88.2% $d_3$, 7.8% $d_2$, 4.0% $d_1$, 1d, 85.5% $d_4$, 10.0% $d_3$, 4.7% $d_2$; 1e, 95% $d_6$, 4% $d_5$; 1f, 91% $d_5$, 9% $d_4$.

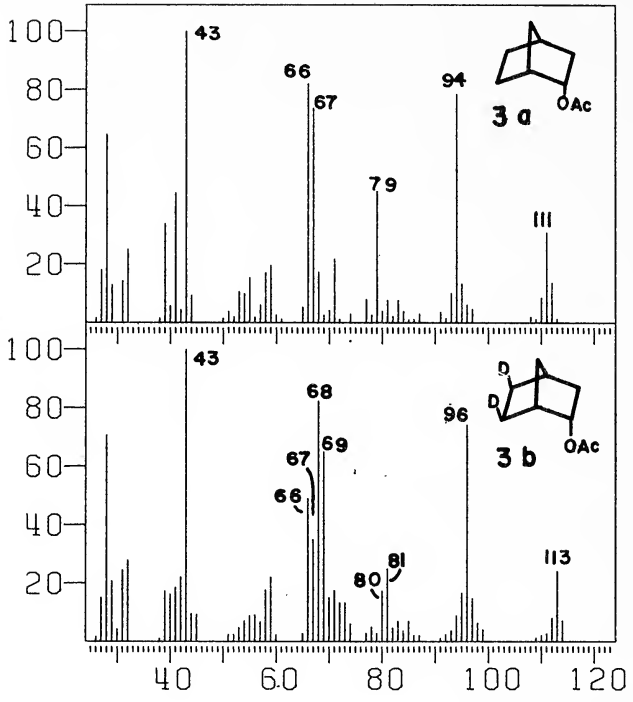



Figure 2. Mass spectra (relative abundance *vs. m/e*) for *endo*-2-norbornyl acetate ( 31a) and deuterio derivative 31b. The origins of peaks specifically identified are discussed in the text.

*Norbornyl Acetates 3a,b*

The synthesis of these derivatives is described in the same source as for the norcamphor derivatives (Marshall and Walter, 1974). Owing to the very low intensity of the parent peaks for 3a,b, an isotopic composition was not determined; however, 3b preceeds 1b in the synthetic sequence (Marshall and Walter, 1974) and hence must have an isotopic purity equal to or greater than that for 1b ( $\geq$ 86.6% $d_2$). Analysis of the norbornene peak(*m/e* 94) in 3a,b indicated 87% $d_2$.

*Mass Spectra*

Mass spectra were run on a Hitachi-Perkin-Elmer RMU-6E mass spectrometer at 70 ev at 2-3 x $10^{-6}$ torr.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Robert A. Welch Foundation, Grant No. B-325, and North Texas State University Faculty Research for financial support of this research. The assistance of Mr. Robert Owens in obtaining the mass spectra of 1a-f is also gratefully acknowledged.

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# CHROMATOGRAPHIC STUDIES OF INTERACTION COEFFICIENTS OF BENZOPHENONE AND BENZHYDROL WITH VARIOUS COMPOUNDS[1]

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ABSTRACT

The interaction coefficients of benzophenone and benzhydrol with various test compounds were determined. The stationary chromatographic columns were Fluoropak 80 supported benzophenone, benzhydrol, and mixtures of the two compounds. The test compounds included acetic acid, allyl ether, 1-butanol, 1-decene, ethanol, heptanal, methyl acetate, and pyrrole. The interaction coefficients were determined as compared to a hypothetical *n*-alkane of the same molecular weight as the test compound. The interaction coefficients of column mixtures tend to be weighted averages of the coefficients obtained with columns of the pure compounds.

The results indicate that the interaction coefficients might be useful in the characterization of mixtures as to properties such as polarity. However, they do not promise to give qualitative or quantitative data related to functional groups of a mixture.

## INTRODUCTION

Retention behavior related to solute-solvent interactions in gas-liquid chromatography (GLC) depends on chemical properties of functional groups (Wehrli and Kovats, 1959; James and Martin, 1954; and Heubner, 1962). Kovats (1958) proposed a method of using retention indices for expressing qualitative GLC values.

Equivalent chain-length numbers which are parameters similar to the retention index have been used to characterize fatty acids (Miwa, *et al.*, 1960). A combination of 2 values, one from a polar and the other from a non-polar stationary column, was sufficient for characterization.

Brown (1963) used retention volumes of 3 selected compounds to classify stationary phases according to their polarity and behavior as electron donors or acceptors. Brown suggested the elimination of hydrocarbon chain length to study the behavior of functional groups.

[1]This publication is a part of the M.A. thesis of Terry K. Henson, whose present address is E. I. du Pont de Nemours and Company, Textile Fibers Division, Chattanooga, TN 38403.

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Rohrschneider (1966) characterized stationary liquids by retention index differences of 5 selected compounds. The method was based on additivity of intermolecular forces. Kaiser (1963) suggests that the greater retention times will result from the greater sum of interacting forces.

Davis, *et al.*, (1966) proposed the technique of inverse gas-liquid chromatography (IGLC) to characterize asphalts. The primary interest is placed on the stationary phase rather than the mobile phase as in conventional GLC. The asphalt was placed on the support column and characterized by determining corrected retention volumes of a series of selected test compounds of various functional groups. The retention data were quantified by relating the retention volume of the test compound to the behavior of *n*-alkanes of the same molecular weight. The retention data were expressed as interaciton coefficients ($I_p$) and were calculated using the equation

$$I_p = [\log V_R^\circ \text{ (test compound)} - \log V_R^\circ \text{ (hypothetical } n\text{-alkane)}] \times 100 \qquad (1)$$

where $V_R^\circ$ is the corrected retention volume.

Air is injected with the test compound, and the retention time ($t_R$) is measured from the appearance of the air peak to the appearance of the peak of the test compound. $V_R^\circ$ is determined from $t_R$ and the flow rate ($F$):

$$V_R^\circ = t_R F. \qquad (2)$$

The values of $I_p$ depend on the interactions of test compounds with the functionality of the asphalt and were useful in showing differences among asphalts but did not provide definite conclusions on the composition of the asphalts.

The correlation of the interaction coefficients of various functional groups of pure compounds with the interaction coefficients of mixtures was studied for benzhydrol, benzophenone and mixtures of these 2 compounds. These compounds are similar except for the functional groups, and any differences in interaction coefficients with a specific test compound would be due to the interaction with the hydroxyl group of the benzhydrol or the carbonyl group of the benzophenone. The study was to determine if qualitative and/or quantitative data could be derived for functional groups in mixtures of organic compounds.

The vapor pressure of the stationary phase should not exceed 0.5 Torr at the column temperature or the stationary phase would bleed from the column (Kaiser, 1963). Benzhydrol and benzophenone and mixtures of these 2 compounds yielded a stable stationary phase for the column.

## EXPERIMENTAL

The procedure was similar to that of Davis, *et al.*, (1966). All retention data were obtained on a F&M Model 700 gas chromatograph equipped with a thermal conductivity detector and a Leeds and Northrup Speedomax G recorder.

Fluoropak 80 was used as the solid support, and the test compounds listed in Table 1 were of ACS reagent grade or better. Benzhydrol, benzophenone, and mixtures of these 2 compounds were used as the stationary phase. The total

TABLE 1

Interaction Coefficients of Various Systems

| Test Compounds | Interaction Coefficients ($I_p$) | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Acetic Acid | 163.1 | 164.5 | 176.5 | 187.1 | 191.7 |
| Allyl Ether | 45.5 | 49.1 | 52.0 | 57.7 | 60.3 |
| 1-Butanol | 117.5 | 119.2 | 131.7 | 137.1 | 143.3 |
| 1-Decene | 5.8 | 20.0 | 13.3 | 10.2 | 13.5 |
| Ethanol | 110.5 | 114.4 | 125.2 | 132.2 | 139.2 |
| Heptanal | 92.0 | 90.5 | 98.9 | 105.4 | 108.5 |
| Methyl Acetate | 56.9 | 67.7 | 71.0 | 77.2 | 82.5 |
| Pyrrole | 196.9 | 196.1 | 188.7 | 190.5 | 188.1 |

Stationary Phase (weight % supported on Fluoropak 80):
System A - 10.0% Benzophenone
System B - 7.5% Benzophenone - 2.5% Benzhydrol
System C - 5.0% Benzophenone - 5.0% Benzhydrol
System D - 2.5% Benzophenone - 7.5% Benzhydrol
System E - 10.0% Benzhydrol

amount of the 2-component stationary phase was 10% (by weight) of the column material (1 part stationary phase and 9 parts Fluoropak 80). The interaction coefficients of 1-component stationary phases did not show a large deviation when the weight of stationary phase ranged from 5 to 15% of the column mass. With the exception of 1-decene, the deviation was within 10% of the $I_p$ at a stationary phase of 10% of the column mass. Therefore, a stationary phase of 10% of column mass was selected. The packing was prepared by making a homogeneous slurry of Fluoropak 80 and the desired amount of stationary phase dissolved in a volatile solvent. The slurry was spread out in a shallow pan and allowed to dry overnight (@ 50 C). Four-ft columns were prepared from 1/4-in diameter aluminum tubing. The column temperature was 100 C, and helium was the carrier gas. The column was maintained at 100 C with continual helium flow for a minimum of 2 hrs before the injection of the test compounds. The volume of the test compound was 0.5 $\mu$l.

$V^{\circ}_R$ is obtained for a series of *n*-alkanes and the test compounds for each stationary column. $I_p$ is calculated by Equation (1).

## RESULTS AND DISCUSSION

The test compounds which gave different interaction coefficients for benzophenone and benzhydrol are given in Table 1. The interaction coefficients are

listed for the stationary columns of pure compounds and those which are mixtures of benzophenone and benzhydrol. Each column contains 90% inert support (Fluoropak 80) and 10% stationary phase. If the column contains 2% benzhydrol, then it is 8% benzophenone. Test compounds used which gave the same interaction coefficients are not included in Table 1. These compounds are benzene, cyclohexane, methylcyclohexane, and toluene. The *n*-alkanes which were used as references are also omitted as these would automatically give interaction coefficients of zero.

The interaction coefficients of the mixtures tend to be weighted averages of those of the pure stationary phases. This is shown for representative examples in Figs. 1 and 2. The molecular weights of the 2 components of the stationary

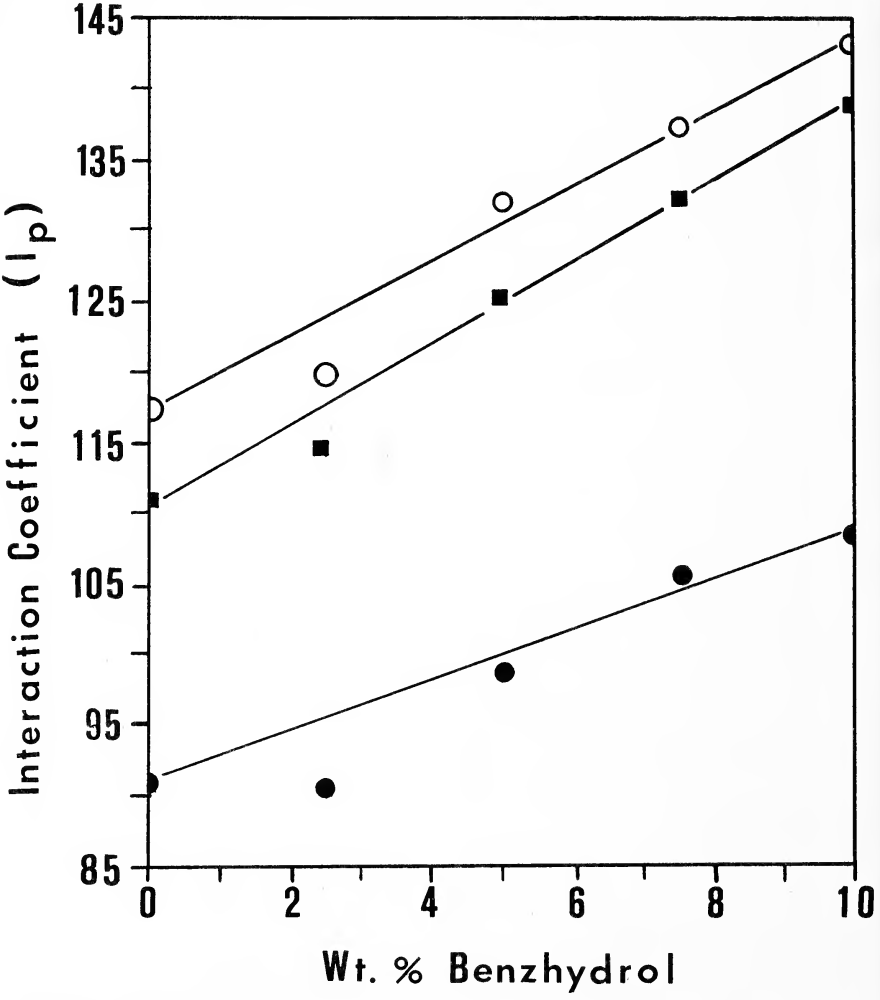


Figure 1. Dependence of interaction coefficient ($I_p$) with the composition of the stationary column. Legend of test compounds: ■ Ethanol, ○ 1-Butanol, ● Heptanal.

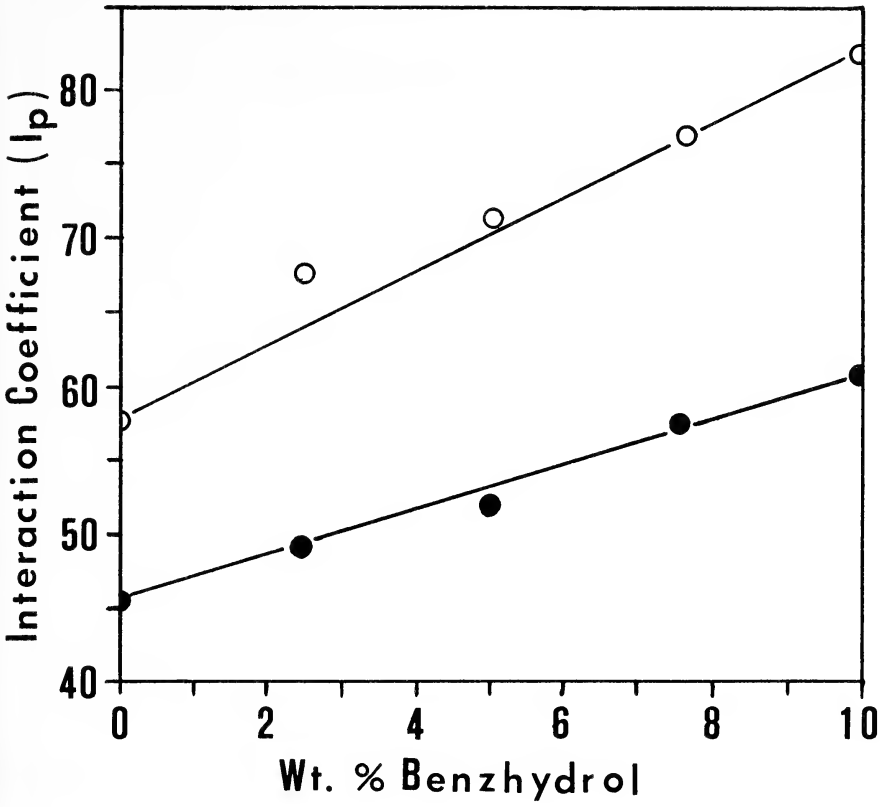


Figure 2. Dependence of interaction coefficient ($I_p$) with the composition of the stationary column. Legend of test compounds: ● Allyl ether, ○ Methyl acetate.

phase are approximately equal (182.2 for benzophenone and 184.2 for benzhydrol). If the weight % of the stationary phase is 2.5% benzhydrol then the mole fraction of benzhydrol in the stationary phase is 0.25, if 5.0% the mole fraction is 0.5, etc. Therefore, the % by weight of one component of the column is linearly proportional to its mole fraction of the stationary phase. The lines drawn in Figs. 1 and 2 are the theoretical lines if the interaction coefficients were linearly dependent on the composition of the stationary phase. The experimental values of $I_p$ are reproducible within 5%. The interaction coefficients of the 2-component stationary phases seem to be more of a weighted average related to the composition of the stationary phase rather than the sum of interacting forces as might be expected from previous works of Rohrschneider (1966) and Kaiser (1953).

These results indicate that interaction coefficients might be useful in the characterization of mixtures as to properties such as polarity. However, they do not indicate that this is a method for obtaining qualitative or quantitative information related to functional groups present in such complex mixtures as petroleum asphalts.

ACKNOWLEDGEMENT

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# THE TEMPERATURE OF MAXIMUM DENSITY OF DEUTERIUM OXIDE IN CAPILLARIES[1]

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ABSTRACT

In earlier work we have described the discovery of a shift in the temperature of maximum density (TMD) of water in fine capillaries.

We have applied the same technique to heavy water (deuterium oxide) and we find a similar shift in the TMD from a value of 11.7 C for bulk $D_2O$ to 5.8 C for $D_2O$ in a quartz capillary of a diameter of 2 micrometers ($\mu$m).

## INTRODUCTION

The temperature of maximum density (TMD) of water has been shown to change from the normal value of 4.0 C in bulk water to a temperature of -0.3 C in a capillary 4 $\mu$m (Schufle and Venugopalan, 1967; Muller and Schufle, 1968; Schufle and Huang, 1972). It was of interest to determine if a similar effect was observed with deuterium oxide which exhibits a TMD at 11.4 C ± 0.3 C.

### *Normal TMD for $D_2O$*

The TMD for $D_2O$ is given in the Landolt-Bornstein (1950) Tables as 11.6 C. However, when the same data was treated by our computerized, least-squares method (Muller and Schufle, 1968) for determining TMD it produced a value of 11.1 C. We then consulted the original data of G. N. Lewis and R. T. MacDonald (1933), who give a value for the TMD of "about 11.6 C." The computer treatment of this data yielded a value for the TMD of 11.4 C. Kell (1967) gives a value of 11.185 C. Finally, the value determined by the present authors in their own apparatus described in Schufle and Venugopalan (1967) on $D_2O$ in a 0.5 mm diameter capillary was 11.7 C. We believe the best value may be somewhere around 11.2 to 11.4 C. Karasev, *et al.*, (1971) have noted that $D_2O$ has a less pronounced maximum than $H_2O$.

[1] Paper delivered at meeting of Southwestern and Rocky Mountain Division of American Association for the Advancement of Science, Laramie, Wyoming, 24 April 1974. Part of the work was done by Mr. Hao as part of the requirements for the Master of Science degree in Chemistry at New Mexico Highlands University.

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*Determination of TMD in Capillaries*

The variation of volume with temperature was measured for 99.90% $D_2O$. The $D_2O$ was distilled *in vacuo* until a resistivity of 0.7 megohm-cm was attained. The capillaries were filled with $D_2O$ and sealed immediately. Quartz capillaries of diameters 500 $\mu$m, 20 $\mu$m, 10 $\mu$m, 7 $\mu$m, and 2 $\mu$m were filled at the same time. The capillaries were then introduced into the apparatus described in Schufle and Venugopalan (1967) and the length of the column of liquid measured for temperatures from approximately 40 C down to temperatures as low as -36 C for the 7 $\mu$m capillary. Liquid $H_2O$ was carried to -40 C in a 4 $\mu$m capillary (Schufle and Venugopalan, 1967), but with liquid $D_2O$ we were able to reach only -36 C before freezing occurred spontaneously.

The following results (Table 1) were obtained, given in terms of the coefficients of the polynomial of degree 3 of the form:

$$1/L = A(0) + A(1)t + A(2)t^2 + A(3)t^3 \qquad (1)$$

where L is the length of the water column, t is the temperature, and the TMD is the temperature of maximum density obtained by the computer from the experimental data.

TABLE 1

Coefficients for Equation (1).

| Cap. Diam ($\mu$m) | 500 | 20 | 10 | 7 | 2 |
|---|---|---|---|---|---|
| 10 A(0) | 1.893385 | 2.145448 | 2.021029 | 2.094744 | 1.978843 |
| $10^5$ A(1) | 4.055660 | 3.832834 | 3.110212 | 4.917084 | 2.590978 |
| $10^6$ A(2) | -1.633188 | -2.460285 | -2.679592 | -4.333409 | -2.487299 |
| $10^8$ A(3) | -0.568127 | 2.375391 | 3.454090 | 4.274537 | 3.082243 |
| TMD | 11.7 C | 9.0 C | 6.7 C | 6.3 C | 5.8 C |

## DISCUSSION OF RESULTS

The data show a continued shift of the TMD for $D_2O$ from the normal value of about 11.4 C for "bulk" $D_2O$ down to 5.8 C for the smallest capillary with a diameter of 2 $\mu$m. This corresponds to the results previously obtained for ordinary $H_2O$ in Schufle and Venugopalan (1967), Muller and Schufle (1968) and Schufle and Huang (1972).

The model we have proposed (Schufle, *et al.*, 1976) is that of "vicinal water," a layer of water in the vicinity of the surface. We have found that the thickness of the vicinal layer may be of the order of 1 $\mu$m thick. As the water droplet, or the capillary containing water, becomes of smaller diameter the surface layer becomes of relatively greater importance. When the diameter reaches the order of a few micrometers the properties exhibited by the water are principally those of the vicinal layer rather than those of bulk water. This is an alternative model for explaining such anomalous behavior as that described by Derjaguin (1970).

In our model it is proposed that the TMD of vicinal water is at a lower temperature than that of ordinary water. The model seems to be true for $D_2O$ as well as $H_2O$.

It is of interest to note that Karasev, *et al.*, (1971) have shifted their emphasis from their earlier model of anomalous bulk behavior to one of anomalous behavior in small pores. For example they have found a shift in the TMD for $H_2O$ to a temperature below 0 C, and for $D_2O$ to a temperature of 6 C. Derjaguin's abstract of this article reads as follows:

> "Water in bodies with small pores is found not to have a density maximum at +4 C. Heavy water under the same conditions gives a less pronounced maximum, which is shifted towards lower temperatures. Water released from the pores is found to have the properties of ordinary bulk water."

The results of this present work confirm these conclusions of Derjaguin with respect to the shift in TMD for $D_2O$ from 11.4 C to about 6.0 C, for water in small capillaries.

Derjaguin's data give a TMD of 6.2 C for $D_2O$ in small pores when his data are treated by our computer analysis (Muller and Schufle, 1968).

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*EQUUS TAU* OWEN FROM THE PLEISTOCENE OF MITCHELL COUNTY, TEXAS — In the course of compiling records of the fossil remains of small horses found in Pleistocene deposits in North America, I was impressed by the resemblance of the figure of the lower jaw referred to *Equus littoralis* Hay by Hay and Cook (1930, *Proc. Colorado Mus. Nat. Hist.*, 9:4, Plate 2) to the lower jaw (FC 672), from Aguascalientes, Mexico, referred to *Equus tau* (Mooser and Dalquest, 1975, *J. Mamm.*, 56:781, Fig. 6). Dr. K. Don Lindsey loaned me the fossil (Denver Museum of Natural History Number 616), found in the "bison quarry" on Lone Wolf Creek, near Colorado, Texas (for details of this site and fauna see Cook, 1925, *Sci.*, 62:459; and 1926, *Sci. Amer.*, 11:334). Direct comparison of the fossil with the referred jaw of *Equus tau,* from Mexico, shows that both belong to the same species.

The rami of the 2 jaws are of the same size and shape. The pattern of the enamel in the teeth of the specimens is similar except that the teeth of the specimen from Texas are more worn, and the metastylids and metaconids appear more rounded. The slight differences in measurements of the 2 specimens are less than the individual variation to be expected between 2 specimens of a single species of extinct horse.

The measurements that follow are in mm, and those of the Mexican horse are in parentheses. Depth of ramus under P3, 58.3 (58.6). Length P2-M2 (M3 missing in the Texas fossil), 101.2 (100.5). Individual teeth, anteroposterior diameter followed by transverse diameter (measurements of the Texas fossil are from Hay and Cook, *loc. cit.*, p. 14): P2, 22 x 12.5 (24.7 x 11.4); P3, 20 x 13 (20.2 x 12.6); P4, 20 x 14 (19.8 x 12.6); M1, 18 x 12.5 (19.4 x 11.2); M2, 19 x 11.5 (17.2 x 11.0)

The jaw from Mitchell County, Texas, is referable to *Equus tau,* and apparently constitutes the first definite record of this species for the State of Texas.—*Walter W. Dalquest, Department of Biology, Midwestern State University, Wichita Falls 76307.*

THE STRUCTURE OF THE RETINA IN FOUR SPECIES OF WHISTLING DUCKS, *DENDROCYGNA* — Four of the 8 species of whistling (or tree) ducks, *Dendrocygna,* may be placed in 2 groups on the basis of their ecology. The black-bellied whistling duck, *D. autumnalis,* and the plumed whistling duck, *D. eytoni,* are highly terrestrial; in contrast the fulvous whistling duck, *D bicolor,* and the wandering whistling whistling duck, *D. arcuata,* are highly aquatic. Previous studies have shown anatomical differences between the 2 groups correlated with the 2 niches (Rylander and Bolen, 1970, 1974). The feet and legs, for example, are better adapted for walking in the terrestrial type, and the feeding apparatus is more suited for straining in the aquatic type. What makes the study of these 4 species particularly interesting is that one member of each ecological type, *D. autumnalis* and *D. bicolor,* respectively, is found in North America, including Texas, and the second member of each type is found in Australia. Convergent evolution of characters associated with the 2 niches appears, therefore, to provide the best explanation for the anatomical similarities between ecological counterparts that are so widely separated geographically; a correlation phenogram of these relationships supports this contention (Bolen and Rylander, 1974).

Since the 2 cursorial species as well as the 2 diving species feed commonly at night, it was hypothesized that the proportion of rods to cones in their retinas might be similar, thus supporting the contention that the feeding niche is largely maintained by locomotor and feeding features rather than by differences in visual abilities. A contrary situation was suggested by a previous study that showed that the percentage of rods was greater in the black-bellied whistling duck than in the mallard, *Anas platyrhynchos,* a predominately diurnal species (Wells, *et al.*, 1975); both are species not anatomically equipped for feeding in deep water. This paper reports a histological study of the retinas of the 4 species of whistling ducks that fall into the 2 ecological types described above.

One retina from at least one individual of the 4 species was removed, fixed in 10% formalin, embedded in paraffin, sectioned at 15 microns, and stained with hematoxylin and eosin. Rods and cones were identified on the basis of the location of their nuclei in relation to the

outer nuclear layer (Polyak, 1957). Twenty-five counts of rods and cones in each retina were taken of sample areas (12 x 100 microns) measured with an ocular micrometer. The sample areas were picked at random, but the fovea was avoided. The results indicate no significant differences in the proportion of the rods and cones between the 4 species (Table 1).

TABLE 1

Mean rod and cone counts from the retinas of 4 species of whistling ducks.

| Species | No. Ducks Examined[a] | M[b] | | | | % Rods |
|---|---|---|---|---|---|---|
| | | Rods | Sd | Cones | Sd | |
| *D. autumnalis* | 3 | 33.9 | 3.36 | 17.0 | 1.14 | 65.4 |
| *D. bicolor* | 1 | 41.2 | 3.96 | 21.2 | 3.80 | 66.0 |
| *D. eytoni* | 1 | 39.4 | 5.47 | 20.6 | 2.38 | 65.6 |
| *D. arcuata* | 2 | 44.6 | 8.08 | 19.0 | 0.71 | 70.0 |

[a]Counts from 25 randomly selected areas from each duck's retina.
[b]Rods and cones per square millimeter = M x $10^4$.

We therefore conclude that there are no retinal differences reflecting differences in visual abilities between the cursorial and diving types of whistling ducks, and that sight is not an important feature contributing to the maintenance of the respective niches of these 2 ecological types.

ACKNOWLEDGEMENT

This research was supported in part by the Welder Wildlife Foundation.

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—*Stephen M. Womack, Michael K. Rylander, Dept. of Biological Sciences, Texas Tech University, Lubbock 79409, and Eric G. Bolen, Rob and Bessie Welder Wildlife Foundation, Sinton 78387.*

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## COVER PHOTO

(Top) Lateral view of the head of an adult male *Elaphe obsoleta* (WMM 650) from south of Kerrville, Kerr County, Texas. (Bottom) Lateral view of the head of an adult male *Elaphe bairdi* (REO 687) from near Alpine, Brewster County, Texas.

by R. Earl Olson, pp. 79-84.

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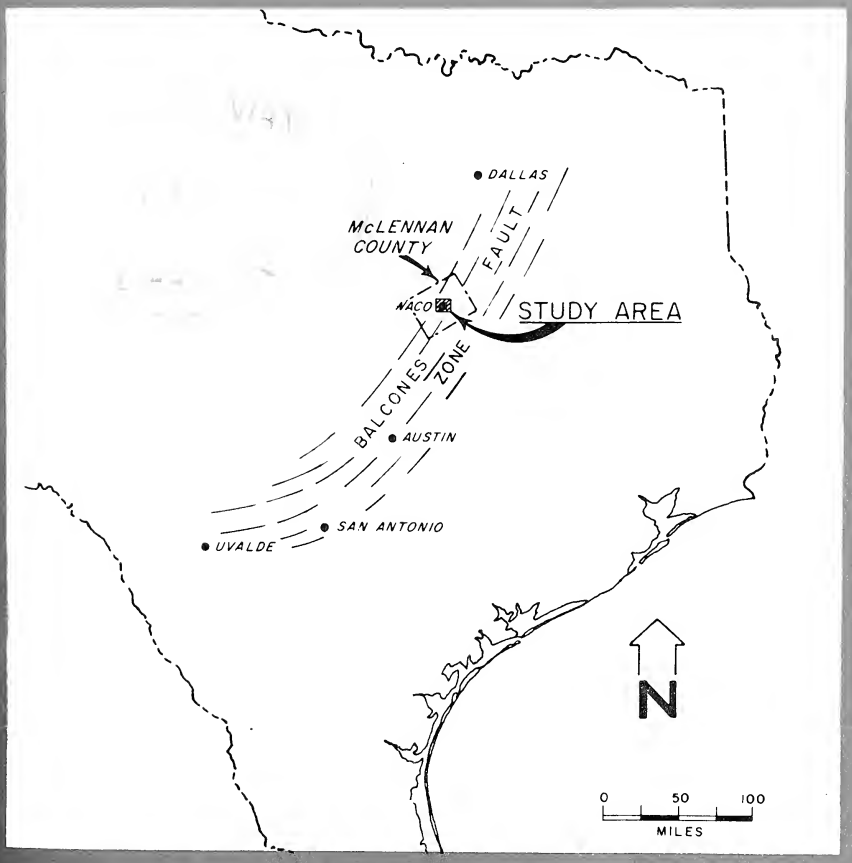

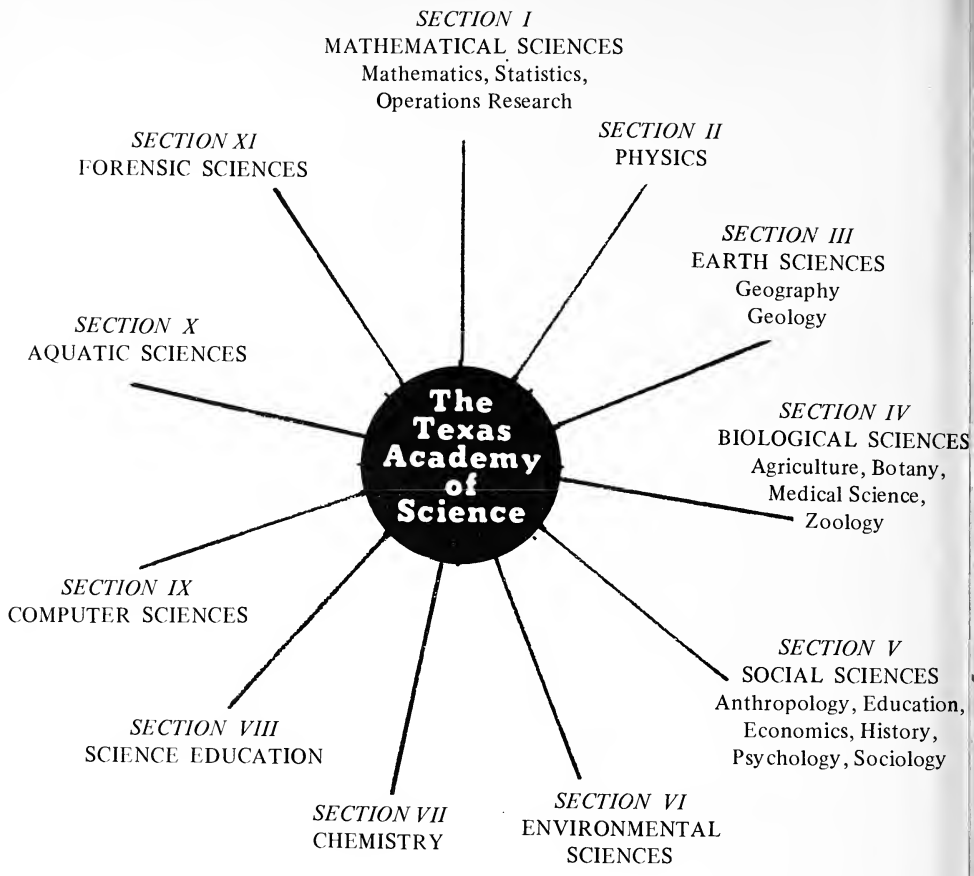


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# LOSS OF EFFICIENCY DUE TO ESTIMATED WEIGHTS IN THE RECOVERY OF INTER-BLOCK INFORMATION

*By* LUISA L. SIA

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ABSTRACT

In the recovery of inter-block information the ratio $\rho$ of the inter-block variance to the intra-block variance is used as weight. $\rho$ being unknown, the ratio of the corresponding variance estimates obtained from the analysis of variance is usually used. It is, however, not unbiased for $\rho$, and often results in biased estimators of treatment effects. Roy and Shah (1962) suggested several alternative estimators of $\rho$ which yielded unbiased estimators of treatment effects. In this paper, the loss of efficiency in the estimation of treatment effects due to sampling error of the weight estimators are calculated using Rao's (1965) delta technique. The same technique is also used for the estimated weights proposed by Kshirsagar (1971) in the recovery of inter-row and inter-column information of a two-way design.

## INTRODUCTION

Let N be the vxb incidence matrix of an experimental design involving v treatments, b blocks of k plots each and r replications. The elements $n_{ij}(i=1,2,...v, j=1,2,...,b)$ of N are 1 or 0 according as the i-th treatment does or does not occur in the j-th block. Assume the design to be connected, then rank C=v-1, where $C=rI_v - \frac{1}{k}NN'$. This implies that there is exactly one eigenvalue of $NN'$ which is equal to rk, and all other eigenvalues are strictly smaller than rk. Suppose rank $NN' = q + 1$, and let $\underline{\xi}_s$, s=1,2,...,q, be a set of orthonormal eigenvectors of $NN'$ corresponding to the q positive eigenvalues $\phi_s$ all smaller than rk, and let $\underline{\xi}_s$, $s=q+1, q+2,...,v-1$, be a set of (v-1)-q orthonormal vectors, each orthogonal to $\underline{\xi}_1, \underline{\xi}_2, ..., \underline{\xi}_q$ and also to $E_{v_1}$. It is well known that the intra-block, inter-block, and combined intra- and inter-block estimates of any treatment contract

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$\underline{\ell}'\underline{\tau}$ are respectively $\underline{\ell}'\hat{\underline{\tau}}$, $\underline{\ell}'\underline{\tau}^*$, and $\underline{\ell}'\tilde{\underline{\tau}}$, where $\hat{\underline{\tau}}$, $\underline{\tau}^*$ and $\tilde{\underline{\tau}}$ are any solutions of the following equations:

$$C\hat{\underline{\tau}} = \underline{Q} \tag{1}$$

$$NN'\underline{\tau}^* = N\underline{B} - \frac{r}{b}GE_{v1} \tag{2}$$

$$(C + \frac{1}{\rho}NN')\tilde{\underline{\tau}} = \underline{Q} + \frac{1}{\rho}(N\underline{B} - \frac{r}{b}GE_{v1}) \tag{3}$$

where

$$\rho = \frac{\sigma^2 + k\sigma_b^2}{\sigma^2} = \frac{\sigma_1^2}{\sigma^2} \tag{4}$$

$$\underline{Q} = \underline{T} - \frac{1}{k}N\underline{B} \tag{5}$$

and $\underline{T}$, $\underline{B}$, G denote the vector of treatment totals, the vector of block totals, and the grand total of a ll the yields respectively. $\sigma^2$ is the intra-block variance/plot, and $\sigma_b^2$ is the variance of the random block effects.

When $\rho$ is known, the minimum variance unbiased estimator of an eigenvector contrast $\underline{\xi}'_s\underline{\tau}$ is given by

$$t_s(\rho) = \underline{\xi}'_s\tilde{\underline{\tau}} \quad \text{for } s = 1, 2,...,q, \tag{6}$$

hence the estimator is a function of $\rho$. For $s = q + 1, q + 2,..., v - 1$, $\underline{\xi}'_s\tilde{\underline{\tau}}$ coincides with $\underline{\xi}'_s\hat{\underline{\tau}}$ and $t_s = \underline{\xi}'_s\hat{\underline{\tau}}$ does not depend on $\rho$. (Kshirsagar and Sia,1974). $\rho$ being usually unknown, an estimate P is often substituted for $\rho$ in (3), and the resulting estimator is denoted by $t_s(P)$. Roy and Shah (1962) proposed estimators of the form

$$P = \frac{aS_1 + \sum_{s=1}^{q} b_s z_s^2}{S_0} + c \tag{7}$$

where $S_0$ is the residual sums of squares from the analysis of variance,

$$z_s = \sqrt{\frac{k}{rk-\phi_s}}\,\underline{Q}'\underline{\xi}_s - \frac{1}{\phi_s}\sqrt{\frac{rk-\phi_s}{k}}\,\underline{B}'N'\underline{\xi}_s \tag{8}$$

$$S_1 = \text{adjusted block S.S.} - \sum_{s=1}^{q}\frac{\phi_s z_s^2}{rk} \tag{9}$$

and a, c, $b_s$ are constants to be determined for each P of specified optimum property. The increase in the sampling error of the treatment effects due to sampling fluctuations of P was shown to be

$$\mathrm{Var}[t_s(P)] = \mathrm{Var}[t_s(\rho)] + d_s \mathrm{Var}(\omega_s) \qquad \text{for } s = 1,2,\ldots,q, \tag{10}$$

where

$$d_s = \frac{k}{\phi_s(\rho + c_s^{-2})} \tag{11}$$

$$\omega_s = \frac{(P-\rho)z_s}{1 + c_s P} \tag{12}$$

$$c_s = \frac{rk-\phi_s}{\phi_s} \tag{13}$$

$S_0$ and $S_1$ are respectively distributed as $\chi^2\sigma^2$ and $\chi^2\sigma_1^2$ with corresponding degrees of freedom

$$c_0 = b(k-1) - (v-1) \tag{14}$$

$$c_1 = (b-1)-q \tag{15}$$

$$z_s \sim \mathrm{NID}(0,\sigma_s^2),\ \sigma_s^2 = \sigma^2 + c_s\sigma_1^2, \qquad \text{for } s = 1, 2,\ldots,q. \tag{16}$$

$S_0$, $S_1$ and $z_s$, s = 1,2,...,q, are all independent.

## LOSS OF EFFICIENCY

Since the variance increment $\mathrm{Var}(\omega_s)$ has not been given in an explicit form, the loss of efficiency due to P cannot be computed. An approximation is obtained in this paper using the delta technique (Rao, 1965).

For algebraic convenience P can be written as

$$P = \frac{cS_0 + aS_1 + u + b_s z_s^2}{S_0} \tag{17}$$

where

$$u = \sum_{i \neq s} b_i z_i^2 \equiv \Sigma' b_i z_i^2 \tag{18}$$

and $\omega_s = \Psi(S_1, S_o, u, z_s)$, a function of $S_1$, $S_o$, u and $z_s$. Using the delta techniques, first take the total differential of $\omega_s$,

$$d\omega_s = \frac{\partial \Psi}{\partial S_1} dS_1 + \frac{\partial \Psi}{\partial S_o} dS_o + \frac{\partial \Psi}{\partial u} du + \frac{\partial \Psi}{\partial z_s} dz_s \qquad (19)$$

where $dS_1$ stands for $S_1 - E(S_1)$, and $\frac{\partial \Psi}{\partial S_1}$ is evaluated at the expected values of $S_1$, $S_o$, u and $z_s$, etc. It is noted that for the proposed P, $E(\omega_s) = 0$. Squaring and taking the expected value yields

$$\mathrm{Var}(\omega_s) \doteq \left(\frac{\partial \Psi}{\partial S_1}\right)^2 \mathrm{Var}(S_1) + \left(\frac{\partial \Psi}{\partial S_o}\right)^2 \mathrm{Var}(S_o)$$
$$+ \left(\frac{\partial \Psi}{\partial u}\right)^2 \mathrm{Var}(u) + \left(\frac{\partial \Psi}{\partial z_s}\right)^2 \mathrm{Var}(z_s), \qquad (20)$$

since all the covariances vanished. We have

$$\mathrm{Var}(\omega_s) \doteq \left(\frac{V_1}{V_2}\right)^2 (\sigma^2 + c_s \sigma_1^2) \qquad (21)$$

where

$$V_1 = (c - \rho)\, e_o + a\rho e_1 + \Sigma' b_i (1 + c_i \rho) \qquad (22)$$

$$V_2 = (1 \mp cc_s) e_o + a\rho c_s e_1 + c_s \Sigma' b_i. \qquad (23)$$

Among the estimators of $\rho$ proposed by Roy and Shah (1962), we consider 3 which will be denoted by $P_1$, $P_2$ and $P_3$ below.

1. To make the classical estimator of $\rho$ unbiased, take

$$a = \frac{k(e_o - 2)}{v(r-1)} \qquad (24)$$

$$c = \frac{-(v-k)}{v(r-1)} \qquad (25)$$

$$b_s = \frac{(e_o - 2)\phi_s}{bk(r-1)} \qquad s = 1, 2, \ldots, q. \qquad (26)$$

P now takes the form

$$P_1 = \left(1 - \frac{2}{e_o}\right) R - \frac{2(v-k)}{v(r-1)e_o} \qquad (27)$$

where R is the classical estimator of $\rho$. $P_1$ is unbiased for $\rho$, and the resulting treatment contrast estimator $t_s(P_1)$ is also unbiased for $t_s(\rho) = \underline{\xi}_s'\underline{\tau}$. Applying (21), the loss of efficiency due to substitution of $P_1$ for $\rho$ is $d_s V_{P_1}(\omega_s)$, where

$$V_{P_1}(\omega_s) \doteqdot \left(\frac{V_{11}}{V_{21}}\right)^2 (\sigma^2 + c_s\sigma_1^2) \tag{28}$$

$$V_{11} = [r(k-v)e_o + (e_o-2)\Sigma'\phi_i]\phi_s + \{vr(r-1)e_o + (e_o-2)[rk(b-2)-\Sigma'\phi_i]\phi_s\rho \tag{29}$$

$$V_{21} = rke_o[r(k-v) + (b-1)\phi_s] + (e_o-2)(rk-\phi_s)\Sigma'\phi_i$$
$$+ (e_o-2)(rk-\phi_s)[rk(b-2)-\Sigma'\phi_i]\rho. \tag{30}$$

2. The variance of the unbiased estimator P of $\rho$ is a quadratic function in $\rho$. When $\rho$ is large, the term involving $\rho^2$ in Var(P) is dominant (Roy and Shah, 1962). The constants can thus be chosen to make the coefficient of $\rho^2$ in Var(P) a minimum, giving

$$a = \frac{3(e_o-2)}{3e_1 + (e_1+2)q} \tag{31}$$

$$c = \frac{-1}{(e_o-2)} \sum_{s=1}^{q} b_s \tag{32}$$

$$b_s = \frac{(e_1+2)a}{3c_s} \tag{33}$$

P now takes the form

$$P_2 = \left[\frac{(e_o-2)}{3e_1 + (e_1+2)q}\right]\left[3S_1 + (e_1+2)\sum_{s=1}^{q}\left(\frac{\phi_s}{rk-\phi_s}\right)\left(z_s^2 + \frac{S_o}{e_o^{-2}}\right)\right]\left(\frac{1}{S_o}\right) \tag{26}$$

$P_2$ is unbiased for $\rho$. Applying (21), the loss of efficiency due to substitution of $P_2$ for $\rho$ is $d_s \cdot V_{P_2}(\omega_s)$, where

$$V_{P_2}(\omega_s) \doteqdot \left(\frac{V_{21}}{V_{22}}\right)^2 (\sigma^2 + c_s\sigma_1^2) \tag{34}$$

$$V_{21} = 2(e_o+2)\sum_{i=1}^{q} c_i^{-1} + (e_oe_1 + 4e_1 + 4q + 2e_1q)[3e_1 + (e_1+2)q]$$
$$\cdot\left(\frac{rk}{\phi_s} - 1\right)^{-1} + [4(e_1-1) + (e_1+2)(e_o+2q)]\rho \tag{35}$$

$$V_{22} = e_o[3e_1 + (e_1 + 2)q] - 2(e_1 + 2)c_s \sum_{i=1}^{q} c_i^{-1} - (e_o - 2)(e_1 + 2)$$
$$+ 3(e_o - 2)c_s[(e_1 + 2)q - 2(e_1 - 1)]\rho. \quad (36)$$

3. Another unbiased estimator of $\rho$ proposed by Roy and Shah is of the form

$$P_3 = (1 - \frac{2}{e_o})\frac{\nu_1}{\nu_o} - \frac{2(v-1)}{e_o(b-1)}(\frac{1}{E} - 1) \quad (37)$$

where

$$E = \frac{(v-1)}{rk} \Big/ \left[\frac{v-1-q}{rk} + \sum_{s=1}^{q} \frac{1}{rk - \phi_s}\right] \quad (38)$$

is Kempthorne's efficiency factor.

$$\nu_o = \frac{S_o}{e_o} \text{ and} \quad (39)$$

$$\nu_1 = \frac{S_o}{e_o(b-1)} \sum_{s=1}^{q} \frac{1}{c_s} + \frac{1}{(b-1)}\left[S_1 + \sum_{s=1}^{q} \frac{z_s^2}{c_s}\right] \quad (40)$$

are unbiased estimates of $\sigma_o^2$ and $\sigma_1^2$ respectively; and they are optimum in the sense that the term involving $\rho^2$ in their respective variances are minimized. Shah (1962) suggested these estimates from intuitive considerations. Applying (21), the loss of efficiency due to substitution of $P_3$ for $\rho$ is $d_s \cdot V_{P_3}(\omega_s)$, where

$$V_{P_3}(\omega_s) \doteqdot \left(\frac{V_{31}}{V_{32}}\right)^2 (\sigma^2 + c_s\sigma_1^2) \quad (41)$$

$$V_{31} = 2(e_o - 3)\sum_{i=1}^{q} c_i^{-1} - (e_o - 2)c_s^{-1} - (b-1)(e_o + 2(b-2))\rho \quad (42)$$

$$V_{32} = (b-2)e_o + 2(e_o - 3)c_s \sum_{i=1}^{q} c_i^{-1} + 2 - (b-2)(e_o - 2)c_s\rho \quad (43)$$

Since $P_1$, $P_2$, and $P_3$ all give unbiased combined inter- and intra-block estimations of an eigenvector contrast; i.e., $t_s(P_1)$, $t_s(P_2)$, and $t_s(P_3)$ are all unbiased for $\underline{\xi}'_s\underline{\tau}$, the choice among the 3 can therefore be guided by the computed loss of efficiency for a particular design.

## APPLICATION OF THESE RESULTS TO SOME DESIGNS

In calculating the loss of efficiency using equations (24), (27) and (29), the parameters needed are q, $e_o$, $e_1$, $\phi_s$, $\Sigma'\phi_i$, $c_s$, and $\Sigma \frac{1}{c_i}$. These are given for 3 designs in this section.

1) BIBD (b, v, r, k, $\lambda$)

$$q = v-1 \tag{44}$$

$$e_o = bk - b - v + 1$$

$$e_1 = b-v \tag{45}$$

$$\phi_s = r-\lambda = \frac{r(v-k)}{v-1} \qquad s = 1, 2,...,v-1 \tag{46}$$

$$\Sigma'\phi_i = \sum_{i \neq s} \phi_i = (v-2)(r-\lambda) = \frac{r(v-2)(v-k)}{v-1} \tag{47}$$

$$c_s = \frac{rk-\phi_s}{\phi_s} = \frac{v(k-1)}{v-k} \qquad s = 1, 2,...,v-1 \tag{48}$$

$$\sum_{i=1}^{q} \frac{1}{c_i} = \frac{(v-1)(v-k)}{v(k-1)} \tag{49}$$

2) GDD (b, v, r, k, $\lambda_1$, $\lambda_2$, m, n)

We consider here a group divisible PBIBD with two associate classes, where the v treatments are divisible into n groups of m treatments each.

$$v = mn \tag{50}$$

$$q = mn-1 \tag{51}$$

$$e_o = bk-b-mn+1 \tag{52}$$

$$e_1 = b-mn \tag{53}$$

$$\phi_s = \begin{cases} r-\lambda_1 & \text{for } s = 1, 2,...,m(n-1) \\ rk-mn\lambda_2 & \text{for } s = m(n-1)+1,...,mn-1. \end{cases} \tag{54}$$

If $s \leq m(n-1)$

$$\Sigma'\phi_i = (mn-2)r + [m(m-2)(n-1)+1]\lambda_1 - (m-1)(mn-m+1)\lambda_2 \tag{55}$$

$$c_s = \frac{r(k-1)-\lambda_1}{r-\lambda_1} \tag{56}$$

$$\sum_{i=1}^{q} \frac{1}{c_i} = \frac{(mn-1)(r-\lambda_1)}{r(k-1)-\lambda_1} \tag{57}$$

If $m(n-1) < s < mn$

$$\Sigma'\phi_i = (mn-2)r+m(m-3)(n-1)\lambda_1 - [m(m-2)(n-1)+2(m-1)]\lambda_2 \tag{58}$$

$$c_s = \frac{mn\lambda_2}{rk-mn\lambda_2} \tag{59}$$

$$\sum_{i=1}^{q} \frac{1}{c_i} \frac{mn(mn-1)\lambda_2}{rk-mn\lambda_2} \tag{60}$$

3) Confounded $s^m$ Factorial Experiment

Consider a factorial experiment in which there are m factors each at s levels (s being the power of a prime). Assume that rank C = v-1, and that out of the $(s^m-1)/(s-1)$ pencils, $n_u$ pencils are confounded in r-u replications and unconfounded in u replications, u = 1, 2,...,r. Then the eigenvalues of C are $\gamma_{ij} = i$, [j = 1, 2,...,$n_i(s-1)$; i = 1, 2,...,r], (Kshirsagar and Sia, 1974). Therefore the eigenvalues of $NN'$ are

$$\phi_{ij} = k(r-i) \qquad \begin{matrix} j = 1, 2,...,n_i(s-1) \\ i = 1, 2,...,r. \end{matrix} \tag{61}$$

Note that the single subscript notation $\phi_s = \phi_{ij}$ may be used with $s = \sum_{u=1}^{i-1} n_j(s-1)+j$; however, a double subscript is more convenient in this case.)

In computing the approximate value of $Var(\omega_{pq})$, the following results are needed:

$$q = s^m - 1 \tag{62}$$

$$e_o = bk-b-s^m + 1 \tag{63}$$

$$e_1 = b-s^m \tag{64}$$

$$\Sigma'\phi_{ij} = \sum_{i\neq p} \sum_{j\neq q} \phi_{ij} = \frac{rs^m}{k}(k^2-k+1) - rk - p \tag{65}$$

$$C_{pq} = \frac{p}{1-p} \tag{66}$$

$$\sum_{i=1}^{r}\sum_{j=1}^{n_i(s-1)}\frac{1}{c_{ij}} = 1-s^{m+r(s-1)}\sum_{i=1}^{r}\frac{n_i}{i} \tag{67}$$

EXTENSION TO TWO-WAY DESIGNS

As in one-way designs the precision of the estimates of treatment effects in two-way designs can be increased by the use of information available from inter-row and inter-column comparisons. The ratio $\rho_r = \frac{\sigma^2 + b\sigma_r^2}{\sigma^2}$ of the inter-row variance to the intra-row and column variance, and the ratio $\rho_c = \frac{\sigma^2 + k\sigma_c^2}{\sigma^2}$ of the inter-column variance to the intra-row and column variance play important roles in the combined inter- and intra- estimations of treatment effects. However $\rho_r$ and $\rho_c$ are usually unknown, and the usual estimates of them obtained from an analysis of variance of the data are biased; as a result the final estimates of the treatment effects are, in general, no longer unbiased. Kshirsagar (1971) extended the results of Roy and Shah (1962) for a particular case of the general two-way design; namely, the case in which the C-matrices of the row and column designs have the same eigenvectors. In the following discussions we shall use the same notations used by Kshirsagar in order to make the presentation and cross-referencing easy.

Let F be the C-matrix of the design, L and M be the row and column incidence matrices respectively. It is assumed that rank $LL' = q_r + 1$ and rank $MM' = q_c + 1$. Rank F = v-1 since the design is connected. Denote the common eigenvectors of F, LL′ and MM′ to be $\frac{1}{\sqrt{v}}E_{v1}, \underline{\xi}_1, \ldots, \underline{\xi}_{v-1}$. Then the combined inter- and intra-estimators of an eigenvector contrast is a function of $\rho_r$ and $\rho_c$; namely,

$$\underline{\xi}_s' \underline{t}^*(\rho_r, \rho_c) = \frac{\underline{\xi}_s' \underline{Q} + \frac{1}{\rho_r}\underline{\xi}_s' \underline{Q}_r + \frac{1}{\rho_c}\underline{\xi}_s' \underline{Q}_c}{\phi_s + \left(\frac{1}{u'\rho_r}\right)^{e_s} + \left(\frac{1}{u\rho_c}\right)^{g_s}} \tag{68}$$

where u = number of rows, u′ = number of columns, $\phi_s$, $e_s$, and $g_s$ are the respective eigenvalues of F, LL′ and MM′ corresponding to $\underline{\xi}_s$; $\underline{Q}$, $\underline{Q}_r$, and $\underline{Q}_c$ are the respective vectors of adjusted treatment totals in the intra-row and column, inter-row, and inter-column estimations.

Kshirsagar (1971) proposed estimators of the weights $\rho_r$ and $\rho_c$ with the forms

$$P_r = \frac{aE_r + \sum_{s=1}^{q_r} b_s\omega_s^2}{E_i} + c \tag{69}$$

$$P_c = \frac{h E_c + \sum_{s=1}^{q_c} d_s \chi_s^2}{E_i} + f \tag{70}$$

where

$$\omega_s = \frac{1}{\phi_s}(\underline{\xi}_s'\underline{Q}) - \frac{u'}{e_s}(\underline{\xi}_s'\underline{Q}_r), \tag{71}$$

$$\chi_s = \frac{1}{\phi_s}(\underline{\xi}_s'\underline{Q}) - \frac{u}{g_s}(\underline{\xi}_s'\underline{Q}_c), \tag{72}$$

and the inter-row, inter-column and combined intra-row and column error S.S. are respectively:

$$E_i \sim \chi^2 \sigma^2 \qquad \text{with } \nu = n-v-u-u'+2 \text{ d.f.}, \tag{73}$$

$$E_r \sim \chi^2(\sigma^2 + u'\sigma_r^2) \qquad \text{with } \nu_r = u-q_r-1 \text{ d.f.} \tag{74}$$

$$E_c \sim \chi^2(\sigma^2 + u\sigma_c^2) \qquad \text{with } \nu_c = u'-q_c-1 \text{ d.f.}; \tag{75}$$

a, c, h, f, $b_s$ and $d_s$ are constants to be determined.

Kshirsagar showed that when $P_r$ and $P_c$ are substituted for the unknown weights $\rho_r$ and $\rho_c$ in $\underline{\xi}_s'\underline{t}^*(\rho_r, \rho_c)$, the resulting estimator $\underline{\xi}_s'\underline{t}^*(P_r, P_c)$ is unbiased for the treatment contrast $\underline{\xi}_s'\underline{t}$; moreover,

$$\mathrm{Var}[\underline{\xi}_s'\underline{t}^*(P_r, P_c)] = \mathrm{Var}[\underline{\xi}_s'\underline{t}^*(\rho_r, \rho_c)] + \mathrm{Var}(z_s) \tag{76}$$

where

$$z_s = \frac{\phi_s e_s}{u'}\left(\frac{1}{\rho_r} - \frac{1}{P_r}\right)\omega_s + \frac{e_s g_s}{uu'\rho_c}\left(\frac{1}{\rho_r} - \frac{1}{P_r}\right)(\omega_s - \chi_s)$$

$$+ \frac{\phi_s g_s}{u}\left(\frac{1}{\rho_c} - \frac{1}{P_c}\right)\chi_s - \frac{e_s g_s}{uu'\rho_r}\left(\frac{1}{\rho_c} - \frac{1}{P_c}\right)(\omega_s - \chi_s) \tag{77}$$

Thus, the effect of substituting $P_r$ and $P_c$ for $\rho_r$ and $\rho_c$ is to increase the variance of $\underline{\xi}_s'\underline{t}^*(\rho_r, \rho_c)$ by $\mathrm{Var}(z_s)$.

When the delta technique is applied to $z_s$, an approximation to its variance is found to be

$$\mathrm{Var}(z_s) = \left[A^2\left(\frac{1}{\phi_s} + \frac{u'\rho_r}{e_s}\right) + B^2\left(\frac{1}{\phi_s} + \frac{u\rho_c}{g_s}\right) + 2AB\left(\frac{1}{\phi_s}\right)\right]\sigma^2 \tag{78}$$

where

$$A = \frac{e_s}{u'}\left[\frac{\phi_s}{\rho_r} - m\nu\left(\phi_s + \frac{g_s}{u\rho_c}\right) + n\nu\left(\frac{g_s}{u\rho_r}\right)\right] \tag{79}$$

$$B = \frac{g_s}{u}\left[\frac{\phi_s}{\rho_c} + m\nu\left(\frac{e_s}{u'\rho_c}\right) - n\nu\left(\phi_s + \frac{e_s}{u'\rho_r}\right)\right] \tag{80}$$

$$m = \left[a\,\nu_r\rho_r + c\nu + \left(\frac{1}{\phi_s} + \frac{u'\rho_r}{e_s}\right)\sum_{i\neq s}^{g_r} b_i\right]^{-1} \tag{81}$$

$$n = \left[h\,\nu_c\rho_c + f\nu + \left(\frac{1}{\phi_s} + \frac{u\rho_c}{g_s}\right)\sum_{i\neq s}^{g_c} d_i\right]^{-1} \tag{82}$$

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# ON THE STATE OF SATURATION OF GROUNDWATER WITH RESPECT TO DISSOLVED CARBONATES, EDWARDS ARTESIAN AQUIFER, SOUTH-CENTRAL TEXAS

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ABSTRACT

Groundwater flowing through the Edwards Limestone in the Balcones fault zone has created extensive cavern systems. Much of the groundwater in the Edwards artesian aquifer has traveled distances exceeding 100 mi. Long distances of flow seemingly should result in water saturated or supersaturated with respect to calcite and dolomite and hence unable to further dissolve host rock. Thus cavern development should be most pronounced near the recharge sites where water is likely to be most undersaturated. Yet caverns in the Edwards aquifer are most extensive near the distal, or discharge-dominated, end of the flow system. The well developed caverns are the result of the general undersaturation of Edwards artesian aquifer water. At least seasonal undersaturation is maintained by introduction of relatively small volumes of flood recharge in Medina, Bexar and Comal Counties to the large groundwater body.

## INTRODUCTION

Groundwater in carbonate aquifers generally flows through void systems that have been created largely by the solvent action of the water on the host rock. In the Balcones fault zone of south-central Texas an extensive system of caverns has been developed in the Lower Cretaceous Edwards Limestone and to a lesser extent in the subjacent Walnut and Glen Rose formations. To evaluate whether cavern development is occurring at present, the state of saturation of the groundwater must be known with respect to the common minerals of older carbonate rocks--calcite and dolomite. If the water emerging at springs is undersaturated then it is aggressive enough to dissolve the rocks it has passed through. If the water is supersaturated at the springs, and for considerable distance into the aquifer from the springs, then dissolution is probably concentrated nearer the recharge areas where the water is still undersaturated.

The purpose of this study was to calculate the saturation of aquifer water samples with respect to calcite and dolomite and then examine their areal trends

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to see if any tentative hypotheses about aquifer development could be made. The water samples examined as to their state of saturation were drawn from the wells and springs whose locations are plotted in Fig. 1 with respect to the boundaries of the Edwards artesian aquifer. Groundwater in the aquifer beneath Bexar and Comal Counties moves dominantly to the northeast into Hays County. For example, groundwater near well 804 in southwestern Bexar County travels toward San Marcos Springs in Hays County. The straight-line distance between these points, which is not to be taken as the actual path of the water, is about 68 mi. Along the way some of the excess water spills out at San Pedro, San Antonio and Comal Springs. The majority of the groundwater in the Edwards aquifer segment shown in Fig. 1 does not come from the local rivers and creeks depicted in Fig. 1. Instead it is derived as underflow within the aquifer from as far as the groundwater divide near Brackettville in Kinney County which is a straight-line distance of about 95 mi to the west of well 804 in Bexar County (Garza, 1962; Arnow, 1963).

## GEOCHEMICAL EVIDENCE ON WATER SATURATION

The key evidence necessary to evaluate the interaction between groundwater and carbonate host rock is the state of saturation of groundwater with respect to calcite and dolomite.

### *Method of Calculating States of Saturation*

Unpublished chemical analyses of spring waters in Comal, Bexar and Hays Counties and of well waters in Bexar County were used in this study; all had pH, bicarbonate concentration, temperature, and sometimes specific conductance determined in the field. The water samples selected for analysis all had a close balance of charges between anions and cations. Ionic strengths (I) were calculated both by taking half the sum of the molalities multiplied by the ion charge squared and by the empirical relationship of Jacobson and Langmuir (1970) where I equals $1.88 \times 10^{-5}$ times the specific conductance. Both methods of calculating ionic strength yielded the same activity coefficients from Figure 4.5 of Garrels and Christ (1965).

The method of calculating the state of saturation of water with respect to calcite and dolomite followed the procedure in case 3, chap 3, (Garrels and Christ, 1965). Solubility constants for calcite and dolomite were adjusted for water temperature according to the values listed in Table 3.2 (Garrels and Christ, 1965) and Table 1 (Thrailkill, 1972), respectively.

The observed and calculated molalities of calcium and magnesium were compared using the calcite saturation index ($SI_C$) and the dolomite saturation index ($SI_D$). $SI_C$ and $SI_D$ are defined as:

$$SI_C = \frac{[Ca^{++}]\ [CO_3{}^{--}]\ \text{observed}}{K_{Calcite}\ \text{calculated}}$$

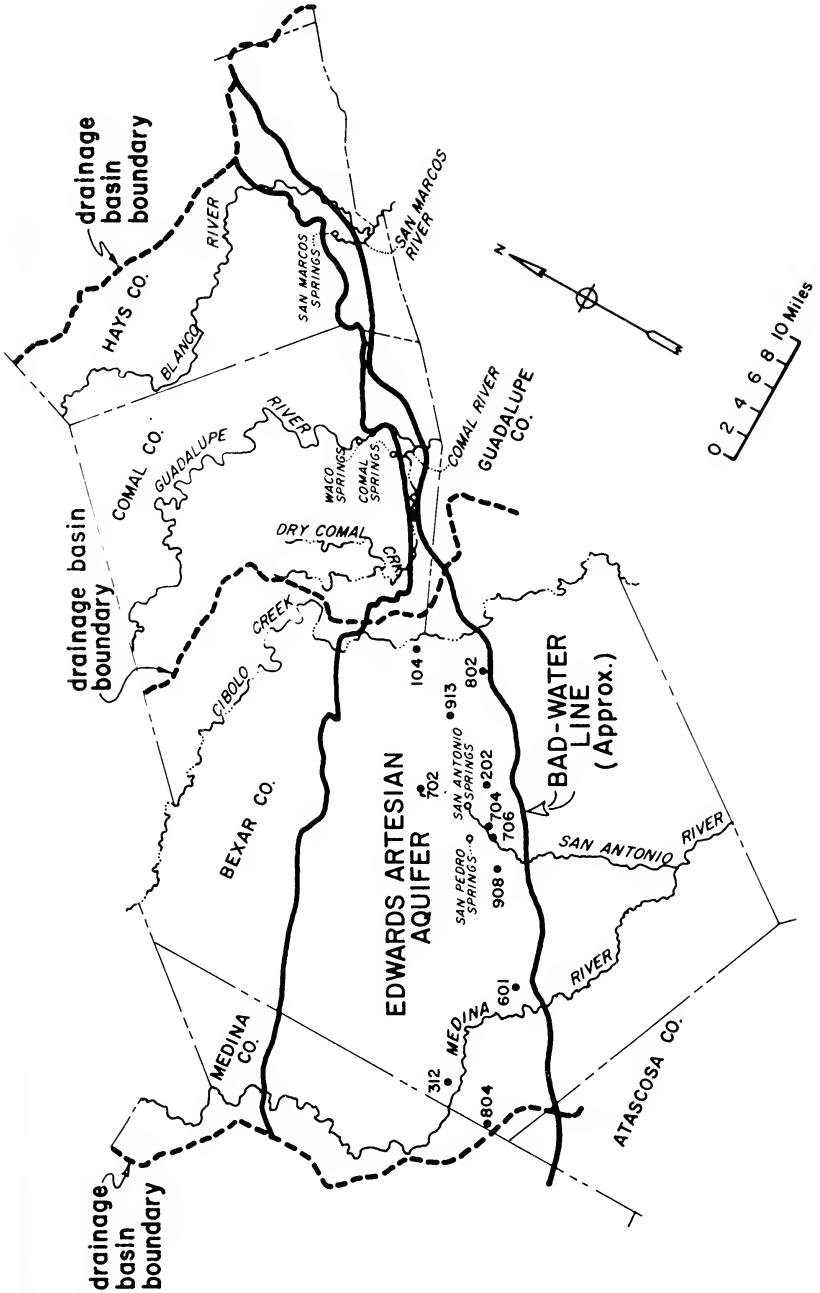


Figure 1. Map of major hydrologic features in Bexar, Comal, and parts of Medina and Hays Counties. Well location numbers are the last 3 digits of the identification numbers in Table 1. Base map for this figure was adapted from Alexander, *et al.*, (1964).

$$SI_D = \frac{[Ca^{++}]\ [Mg^{++}]\ [CO_3^{--}]^2 \text{ observed}}{K_{Dolomite} \text{ calculated}}$$

Negative values of $SI_C$ and $SI_D$ indicate the water samples are undersaturated and positive values show the water is supersaturated.

The saturation indices listed in Table 1 slightly overestimate the water saturation because the molalities of ion pairs were not subtracted. Although the preponderance of water samples in this study were undersaturated, the application of ion-pairing corrections would lower the level of saturation slightly further.

*Results of Water Saturation Calculations*

Examination of the data in Table 1 shows than no entirely consistent generalizations are possible. One area where care must be exercised is in comparing saturation indices of waters drawn from the aquifer on different days, months, and years. Seasonal variations in the state of saturation of groundwater in carbonate terranes have been demonstrated in Pennsylvania by Shuster and White (1971) and in Kentucky by Thrailkill (1972). Thus the only direct comparisons that can be made are between water samples collected at the same time. Notice, for instance, that the least saturated samples were all collected in mid-December 1974 and that most of the supersaturated samples were collected in late March, 1972. It would be most instructive to be able to examine the chemical analyses of a large number of samples extracted from throughout the aquifer in as short a time as possible.

Despite the inadequacies of the presently available data, which are from an aquifer that has non-homogeneous porosity development and anisotropic groundwater flow, some significant hypotheses may be suggested with caution. Inspection of the chemical analyses listed in Table 1 shows that the groundwater and spring water from the Edwards aquifer are predominately undersaturated. Several additional imperfect generalizations may be drawn from these data: 1) water nearer the southeastern boundary, i.e., the bad-water line, of the aquifer is more saturated, 2) concentration of $Ca^{++}$ and $HCO_3^-$ may increase toward the northeast, 3) higher temperature water is more saturated, 4) water from lower yield wells is more saturated, and 5) spring water is the least saturated.

One difficult-to-assess variable that bears directly on the foregoing generalizations is the size of the caverns intersected by a given well and their significance in the overall pattern of groundwater movement. Groundwater flowing rather rapidly through interconnected large openings is likely to be less saturated than slower-moving water wending its way through less direct, smaller openings. This concept may be of value in explaining the location of some of the more saturated groundwater samples. Water from wells near the bad-water line seems to comprise more than a proportional share of the saturated samples. The bad-water boundary, on the down-dip side of the Edwards aquifer, is a dissolution-engrained bypass boundary that marks the original extent of groundwater drawn to the

TABLE 1

Analyses of Well and Spring Waters, Bexar, Comal and Hays Counties

| Well[1] or Spring | $SI_C$ | $SI_D$ | Calcium (mg/1) | Magnesium (mg/1) | Bicarbonate (mg/1) | Date collected | Water temperature | Depth (feet) | Well yield (gpm) |
|---|---|---|---|---|---|---|---|---|---|
| AY-68-42-804 | +0.15 | +0.48 | 63 | 16 | 242 | 3-23-72 | 27.5 C | 2301 | 1200 |
| AY-68-42-312 | -0.11 | -0.16 | 68 | 14 | 248 | 3-23-72 | 25 | 1368 | 1200 |
| AY-68-43-601 | -0.08 | 0 | 66 | 16 | 251 | 9-04-73 | 27 | 1909 | 3000 |
| AY-68-36-908 | -0.14 | -0.06 | 65 | 17 | 248 | 12-07-73 | 27 | 1708 | 2000 |
| AY-68-37-704 | -0.17 | +0.06 | 66 | 22 | 251 | 12-07-73 | 27.5 | 1617 | 800 |
| AY-68-37-706 | -0.21 | -0.12 | 70 | 20 | 246 | 12-07-73 | 27 | 1521 | 1000 |
| San Pedro Springs | -0.29 | -0.52 | 72 | 15 | 256 | 11-23-73 | 24 | -- | -- |
| San Antonio Springs | -0.20 | -0.32 | 76 | 16 | 267 | 11-23-73 | 24 | -- | -- |
| AY-68-29-702 | -0.02 | -0.22 | 89 | 14 | 296 | 3-22-72 | 22.5 | 872 | 2650 |
| AY-68-37-202 | +0.03 | +0.20 | 66 | 15 | 248 | 3-22-72 | 25.5 | 702 | 4000 |
| AY-68-29-913 | -0.12 | -0.16 | 70 | 15 | 260 | 3-22-72 | 25 | 784 | 1300 |
| AY-68-30-802 | +0.03 | +0.24 | 68 | 17 | 244 | 3-22-72 | 27.5 | 750 | 200 |
| AY-68-30-104 | -0.19 | -0.40 | 94 | 17 | 326 | 3-23-72 | 22 | 850 | 1600 |
| Waco (Hueco) Springs | -0.54 | -1.42 | 97 | 13 | 342 | 12-16-74 | 21.5 | -- | -- |
| Comal Springs | -0.78 | -1.54 | 77 | 16 | 283 | 12-16-74 | 23.5 | -- | -- |
| Comal Springs | -0.06 | -0.04 | 80 | 16 | 283 | 5-15-73 | 23.5 | -- | -- |
| San Marcos Springs | -0.64 | -1.24 | 86 | 17 | 313 | 12-16-74 | 22 | -- | -- |
| San Marcos Springs | -0.05 | -0.06 | 86 | 17 | 306 | 5-15-73 | 22 | -- | -- |

[1]Wells located by system of Texas Water Development Board.

earliest discharge sites (Abbott, 1975). As the down-dip portion of the aquifer seems to have received less overall flow it follows that wells nearer the bad-water line are more likely to penetrate smaller, less significant caverns that contain more saturated water.

Wells with the lowest water yields furnished most of the supersaturated water samples. This trend is sharply reversed by the supersaturated state of the water from the highest yield well (202). Remember that even the most saturated waters are only slightly supersaturated. Well 202 is about 2 mi from a salient in the bad-water line and was sampled during late March 1972 when seasonal saturation appears to have been high. At any rate, lower yield wells are probably drawing from lesser developed conduits with slower water flow, increased contact with the wall rock and, hence, higher levels of saturation.

The least saturated waters are those discharged from springs. This observation may be explained by invoking the presence of large conduits leading more or less directly from recharge areas to discharge sites. The large volumes of undersaturated water directed to springs dissolve even larger conduits en route which sets up a self-perpetuating system. As the size of caverns increases, an ever-decreasing percentage of the groundwater is in contact with the walls at any instant. Thus aggressive waters necessary for dissolution tend to be available and their volume, compared to the surface area of the conduit walls, is great.

*Effect of Mixing of Waters*

The occurrence of rock dissolution in the Edwards aquifer may be appreciated by comparing the concentrations of $Ca^{++}$, $Mg^{++}$, and $HCO_3^-$ listed in Table 1 with the positions of the wells and springs on Fig. 1. $Mg^{++}$ shows no systematic changes in concentration but $Ca^{++}$ and $HCO_3^-$ do. The concentration of $Ca^{++}$ in mg/l is generally in the 60's in western Bexar County, in the 70's in central and eastern Bexar County, and in the 80's in Comal and Hays Counties. The increase in $Ca^{++}$ concentration is probably due to dissolution of host rock by undersaturated water moving northeast in the aquifer. This trend is most pronounced in the central portion of the aquifer where water flow is presumably more voluminous. In the vicinity of the bad-water line the $Ca^{++}$ concentrations of water samples are similar which further suggests that water does not circulate as freely there.

The northeastward increase in $HCO_3^-$ concentration parallels the increase in $Ca^{++}$ concentration. $HCO_3^-$ concentrations in mg/l are largely in the 240's in western Bexar County, in the 250's and 260's in central Bexar County, and in the 300's in Hays County.

Considering that the majority of the groundwater in the Edwards aquifer in Bexar and Comal Counties has come in as underflow from the counties to the west (Garza, 1962; Arnow, 1963), it is surprising to find groundwater undersaturated with respect to calcite and dolomite at the distal end of the aquifer. Yet

the least saturated waters found in these analyses were collected from Comal and San Marcos Springs which are over 150 mi from the proximal, or recharge-dominated, end of the aquifer and despite the apparent increase in $Ca^{++}$ and $HCO_3^-$ concentrations through Bexar, Comal and Hays Counties.

The processes that occur to create this unexpected undersaturation have been explained best by Thrailkill (1968). Meteoric water that descends through the soil and unsaturated zones (vadose seepage) may enter the aquifer in a saturated or supersaturated state, but if it is cooled upon entering the main groundwater body or is mixed with groundwater which is in equilibrium with a lower partial pressure of $CO_2$, the net effect is to cause undersaturation in the main water body. The significant process here is the introduction of $CO_2$ into the groundwater body which allows further dissolution to occur.

Probably the most important factor in keeping the main body of groundwater undersaturated is the introduction of large volumes of flood water in Medina, Bexar and Comal Counties that are undersaturated with respect to calcite and dolomite. Arnow (1963) has estimated that about 1/6 of the groundwater in the Edwards aquifer in Bexar County is derived as local recharge from the Medina River, Cibolo Creek and other smaller streams such as Salado and Leon Creeks. George (1952) calculated that about 1/4 to 1/3 of the water flow from Comal Springs is obtained as recharge from the Cibolo and Dry Comal Creek basins; the Guadalupe River flows across the aquifer without significant changes in its discharge. Saturation indices for water in creeks that recharge the aquifer were not calculated because no chemical analyses with field-measured pH were available. However, the concentrations of $Ca^{++}$, $Mg^{++}$, and $HCO_3^-$ are available for surface water samples collected under variable discharge conditions from these creeks (e.g. Land, 1972, Steger, 1973). The bulk of the waters that flow in these creeks have $Ca^{++}$, $Mg^{++}$, and $HCO_3^-$ concentrations that are markedly less than those in the aquifer. During times of low discharge, when the creeks are fed by small springs, the ion concentrations in the water may be higher than in the aquifer. But the stream waters that flow in the high discharge conditions that occur after heavy rains commonly have ion concentrations that are only 1/4 to 1/2 of those in the aquifer (Tables in Land, 1972, and Steger, 1973). More recharge occurs from floods than from base flow. The addition of these high volume, low ionic concentration waters into the Edwards aquifer seems to be the major factor in maintaining a general state of undersaturation.

For purposes of comparison it is interesting to note the ion concentrations in Waco Springs water (Table 1). Although this spring system discharges just 3 mi north of Comal Springs it is not connected to the major aquifer and its recharge area appears to be entirely within Comal County to the west and northwest of the springs (Fig. 1). The mid-December, 1974 analysis showed the spring water had a 1/8 to 1/4 higher concentration of $Ca^{++}$ and $HCO_3^-$ than the waters discharged from the major aquifer at Comal and San Marcos Springs. The Waco Springs are a much simpler system where undersaturated flood waters recharge

a separate but parallel aquifer, flow several mi in a large cavern system without dilution by significant amounts of other recharge, and emerge with higher ion concentrations than the long distance waters discharged at Comal and San Marcos Springs. The higher ion concentrations developed in short flow distances in this local, although large, system are probably attributable in part to the absence of a significant mixing-of-waters effect.

## CONCLUSIONS

Despite straight-line travel distances exceeding 160 mi for some of the groundwater in the Edwards aquifer the groundwater appears to be undersaturated at times. This undersaturation is due partly to large volumes of groundwater flowing in pipe-like voids where little of the water is actually in contact with the host rock, and partly to the mixing effect that occurs with addition of significant volumes of undersaturated recharge. The undersaturated state of some of the groundwater in the aquifer implies that caverns are probably being enlarged during seasonal conditions. Those areas transmitting the greatest amounts of water probably are receiving the greatest amounts of dissolution.

## ACKNOWLEDGEMENTS

This paper was made possible by the chemical analyses of water samples furnished by Robert W. Maclay of the U.S. Geological Survey in San Antonio. Special thanks go to Paul Rettman of the U.S.G.S. for collecting the water samples and making field measurements of pH, specific conductance and bicarbonate concentration. The manuscript was improved by the comments of Charles M. Woodruff, Jr. of the Bureau of Economic Geology at the University of Texas. The figure was drafted by Enos. J. Strawn.

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# FITTING SOIL TEMPERATURE BY A PERIODIC REGRESSION MODEL

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ABSTRACT

A sine curve regression model is presented to which monthly mean soil temperature may be fitted from mean air temperature. The model was separately tested at Nacogdoches, Texas and Kearneysville, West Virginia with predictabilities greater than 98% and standard error of estimates less than 1 C.

## THE PROBLEM

Soil temperature is one of the important factors governing biological, chemical, and physical processes in soil. For example, the rates of organic-matter decomposition and evaporation loss increase with soil temperature, while seed-germination and root-growth are impossible for most species when temperature is less than 40 F. Below freezing there is little biological activity, no chemical weathering, no liquid water movement, and, as a consequence, no soil formation--time stands still for the soil. As Smith, *et al.*, (1964) expressed it, soil comes to life when its temperature exceeds 42 F. For these reasons, mean annual soil temperature (at 50 cm depth) is used as one of the criteria to differentiate soil families in the new U.S. soil classification system (USDA, 1975).

Soil temperature varies in time and space due to the difference in 1) heat flux into the soil, 2) the heat transfer in the soil, and 3) the heat exchange between soil and air. Since soil temperature data are usually available at only a few stations in each state, estimates based on air temperature or other climatological elements become necessary for practical purposes. The Soil Conservation Service (Smith, *et al.*, 1964) estimates annual soil temperature by adding

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2 F (about 1 C) to the annual air temperature. For a farmer or forester, however, monthly temperature is more important than annual as it reflects seasonal patterns of evaporation, transpiration, snowmelt, and plant growth. The objective of this study was to develop a periodic regression model to estimate monthly soil temperature at Nacogdoches, Texas with acceptable accuracy.

## THE PREDICTION MODEL

Heat transfers in soil are governed by the nature of the soil, the type of surface cover, and incoming radiation. For estimating long-term average monthly soil-temperature at a particular station, one can assume that basic soil properties are constant and surface cover varies in a relatively fixed pattern during the year. Thus the seasonal fluctuation of soil temperature is controlled primarily by incoming radiation, which can be indexed as a function of air temperature (TA) and time angle:

$$ST_t = a_0 + a_1 TA_t + a_2 SIN(2\pi t/12) + a_3 Cos(2\pi t/12) + \epsilon_t \qquad (1)$$

where

$ST$ = monthly soil temperature, C,
$2\pi t/12$ = the monthly units of time in a single cycle to angular measure in radians,
$t$ = month, from 0 (January) to 11 (December),
$\epsilon_t$ = error term,

and $a_0$, $a_1$, $a_2$, and $a_3$ are constant. Equation 1 is a sine curve with modification of air temperature. For estimating annual soil temperature ($ST_y$), the Equation becomes

$$ST_y = a_0 + a_1\left(\sum_{t=0}^{t=11} TA_t\right)/12 + \epsilon_y \qquad (2)$$

where $\epsilon_y$ is the error of the predicted annual soil temperature. Once Equation 1 is developed, the only climatological data required for solving the equations are monthly mean air temperatures, TA. Values of the time angle, $2\pi t/12$, for each month are shown in Table 1.

## DATA, ANALYSIS, AND RESULTS

The soil temperature data used in the analysis were observed at the Stephen F. Austin Experimental Forest, 15 miles southwest of Nacogdoches, Texas from May 1957 to September 1959. The soils are loams and sandy loams of the Shubuta series (formerly mapped as Boswell series), a member of the clayey, mixed, thermic family of Aquic Hapludults, supporting a mixed stand of second-growth hardwoods and loblolly-shortleaf pine. The annual rainfall during

TABLE 1

Values of the Monthly Time Angles

| Month | t | Sin(2πt/12) | Cos(2π/12) |
|---|---|---|---|
| January | 0 | 0.0000 | 1.000 |
| February | 1 | 0.5000 | 0.8660 |
| March | 2 | 0.8660 | 0.5000 |
| April | 3 | 1.0000 | -0.0000 |
| May | 4 | 0.8660 | -0.5000 |
| June | 5 | 0.5000 | -0.8660 |
| July | 6 | -0.0000 | -1.0000 |
| August | 7 | -0.5000 | -0.8660 |
| September | 8 | -0.8660 | -0.5000 |
| October | 9 | -1.0000 | 0.0000 |
| November | 10 | -0.8660 | 0.5000 |
| December | 11 | -0.5000 | 0.8660 |

the period was 47.58 in (1209 mm), or 0.46 in (12 mm) below normal (1931-60). Soil temperature was recorded tri-weekly using Colman fiberglass units with thermisters at two different depths (6 and 12 in) in nine 1/10 acre circular plots. Air temperature at 4 ft above the ground was observed at the weather station in the SFA Experimental Forest. The data have been published by Schneider and Stransky (1966).

Equation 1 was employed to fit the 29 monthly soil temperature values for each depth observed at the Experimental Forest. The analysis showed that this equation explained more than 98% of soil-temperature variation, with a standard error of estimates less than 3.7% or 0.69 C (Table 2). Since the thermal capacity, conductivity, and diffusivity of a soil are affected by its moisture content, precipitation (use as an index of soil moisture) was added to Equation 1 in the regression analysis. However, the inclusion of a precipitation variable did not improve the accuracy of the prediction.

TABLE 2

The Constants and Simple Statistics of Equation 1 for Two Different Locations

| Location | Depth (In) | Constants $a_0$ | $a_1$ | $a_2$ | $a_3$ | Statistics[1] $R^2$ | SE,% |
|---|---|---|---|---|---|---|---|
| Nacogdoches, Texas | 6 | 2.82 | 0.91 | -0.84 | 1.49 | 0.987 | 3.5 |
| | 20 | 5.05 | 0.82 | -1.73 | 1.72 | 0.980 | 3.7 |
| Kearneysville, W.V. | 8 | 7.85 | 0.48 | -2.22 | -6.25 | 0.987 | 8.2 |
| | 20 | 9.57 | 0.47 | -3.66 | -4.39 | 0.976 | 8.7 |

[1] $R^2$: Coefficient of multiple determination; SE: standard error of estimate.

The average observed and estimated values at the 6-in depth were plotted in Fig 1 for further illustration. Greatest errors of estimates at the 6- and 12-in levels were -1.26 C and -1.40 C, respectively, both in September 1959. Of the

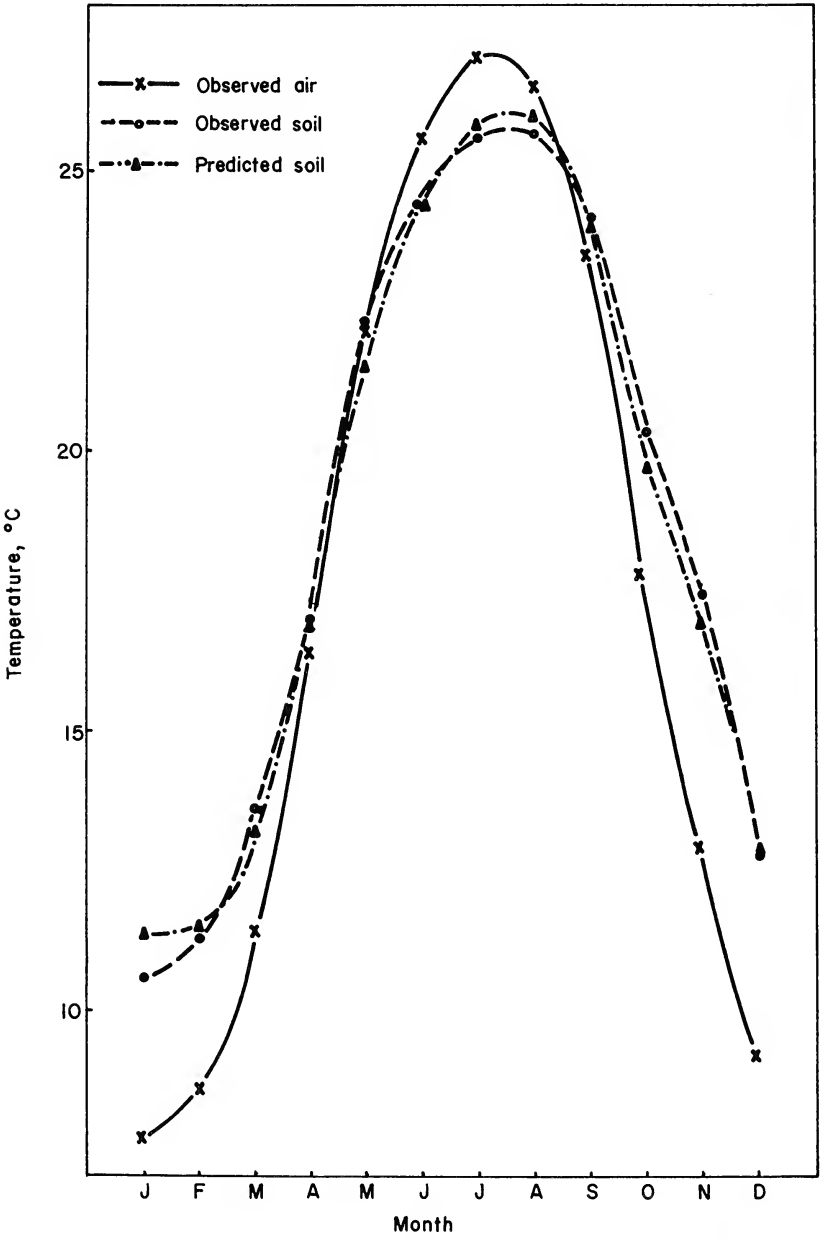


Figure 1. Estimated and observed monthly mean soil temperatures at the 6-in level and the observed air temperatures at the SFA Experimental Forest, Nacogdoches, Texas.

58 estimates, only 4 at the 6-in level and 6 at the 12-in level had errors of estimate greater than ± 1 C. Except for the January soil temperature at the 12-in level in 1958, all the greatest errors were in the summer months (i.e., July, August, and September). Normally the 3 summer months are the driest of the year in Nacogdoches. As soils become drier their thermal capacity tends to be reduced, thus increasing the fluctuations of the soil temperatures. This would account, at least in part, for the greatest errors in estimates of soil temperatures occurring during the summer.

The soil temperatures regime, as shown in Figure 1, at the SFA Experimental Forest followed the general patterns in humid regions described by Chang (1958). Heat Conduction to and from the air-soil interface causes monthly mean soil temperature to increase with depth during winter and to decrease with depth during summer. Soil temperatures at 6 and 12 in were generally warmer than mean air temperature during the colder months and cooler during the warmer months. Temperature differences in colder months, however, were less than in observations made in West Virginia (Chang and Lee, 1977).

## APPLICATION

Applicability of Equation 1 was treated by fitting 36 monthly mean soil temperature values (1963-65) at two depths (8 and 20 in) observed at Kearneysville, West Virginia (Weedfall, *et al.,* 1967). The soil is a Hagerstown series of fine, mixed, mesic family, Typic Hapludalfs. The analysis (Table 2) showed that the $R^2$ values at these two soil levels were greater than 0.97 with an average absolute error less than 7% (0.97 C). The great accuracy suggests that Equation 1 is an applicable model for fitting monthly mean soil temperature data at a particular station. If data are available at sufficient number of stations for spatial analysis, it could be also employed to estimate monthly soil temperatures at unaged sites in a region.

## ACKNOWLEDGEMENTS

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# TRACE FOSSILS FROM THE PECAN GAP FORMATION (UPPER CRETACEOUS), NORTHEAST TEXAS[1]

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ABSTRACT

The Pecan Gap Formation in northeast Texas exhibits varied lithologies and several distinctive sedimentary features that provide significant information concerning the depositional history of the Taylor Group. Trace fossils are the characteristic megafossil and consequently furnish the most promising source of paleoenvironmental data.

Two distinct assemblages of ichnofossils were identified: 1) small *Thalassinoides*, horizontal *Rhizocorallium*, and *Stipsellus* occur within a thinly bedded glauconitic facies; 2) large *Thalassinoides*, vertical *Rhizocorallium, Gyrolithes*(?), *Pseudobilobites*, and numerous vertical burrows are found in a "massive" quartzose calcarenite facies. Both assemblages are indicative of inner shelf deposition. Morphologic differences displayed by these 2 groups of trace fossils probably resulted from local variations in energy as suggested by physical sedimentary features.

## INTRODUCTION

Our knowledge of the Taylor Group in northeast Texas has evolved gradually to a picture of complex stratigraphic relations which consist of a myriad of intercalated facies. It is generally accepted that the Taylor Group is a neritic marine

[1]This work was taken in part from a M.S. thesis in geology completed by the senior author at The University of Texas at Arlington, August, 1976.

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sequence deposited during the Late Cretaceous (Beall, 1964; Powell, 1970). However, a comprehensive depositional model including all local facies has not yet been established.

Presented herein is paleoenvironmental information derived primarily from studies of trace fossils collected at exposures of the Pecan Gap Formation in Collin and Rockwall counties, Texas (Fig. 1). The present writers hope that our results will be integrated with future surface and subsurface studies to develop a complete history for Late Cretaceous sedimentation in northeast Texas.

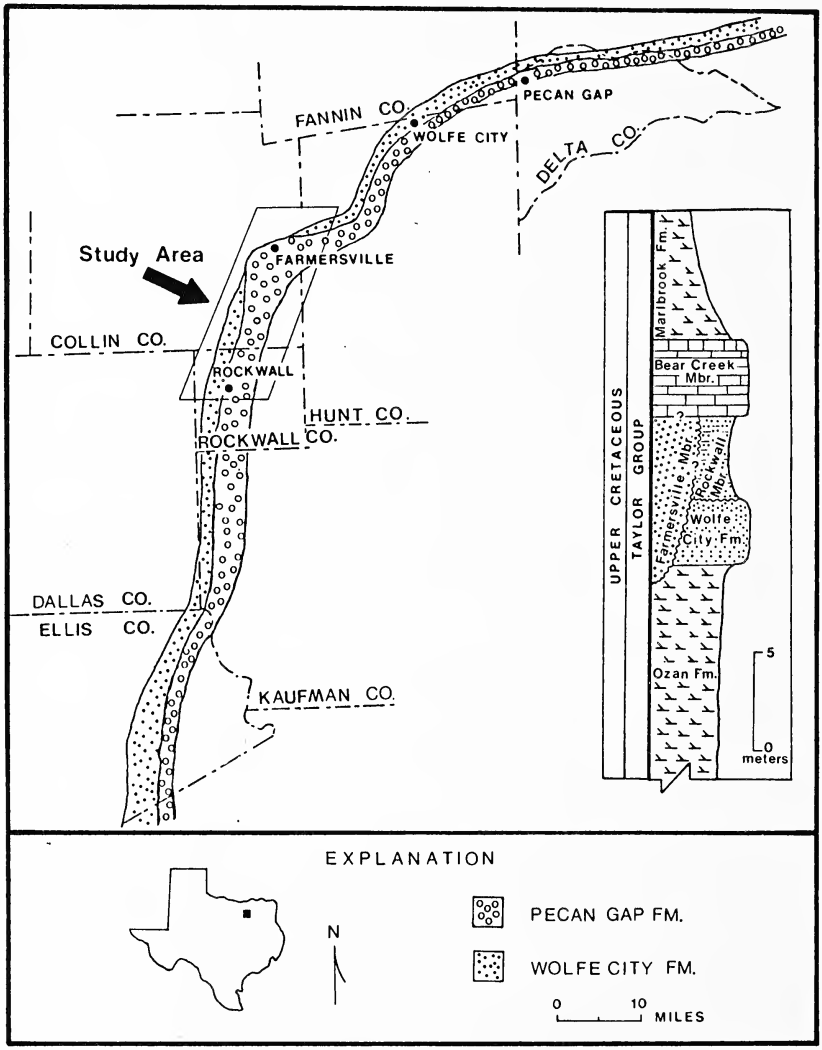



Figure 1. Location of study area and outcrop of the Wolfe City and Pecan Gap formations (Geology modified from Barnes, 1967, 1972).

## GENERAL STRATIGRAPHY

The general stratigraphy of the Taylor Group is essentially as described originally by Stephenson (1918). The Pecan Gap Formation disconformably overlies the Wolfe City Sandstone in its type area and is a relatively thin medial carbonate unit within a thicker, dominantly argillaceous sequence.

Although the Taylor Group can be subdivided into lithologically distinct rock units on a regional scale, localized facies complicate the stratigraphy (Fig.1).

Near Farmersville in Collin County the Pecan Gap changes laterally from a biocalcarenite to a densely burrowed glauconitic and phosphatic calcarenite within a distance of about a mile (Rouse, 1944; Brown, 1949). Brezina (1974) proposed the name "Farmersville Member" for this bioturbated glauconitic facies.

The member is bounded laterally on the north by the Wolfe City Formation (Brezina, 1974) and disconformably overlies the Ozan Formation (Brown, 1949; Dawson, 1976). The basal contact is marked by a discontinuous phosphate conglomerate.

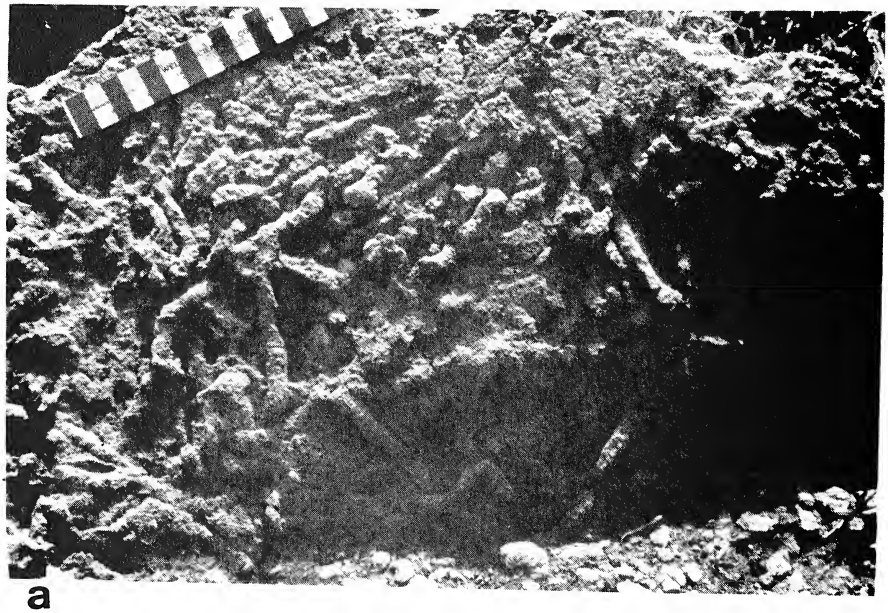
According to Brezina (1974, Fig. 5), the Farmersville Member is laterally juxtaposed with a silty marl that is present in Rockwall and southern Collin counties. Maluf (1975) undertook a study of this area and subsequently divided the Pecan Gap Formation exposed there into the following lithologic units: Rockwall Member (silty marl) and Bear Creek Member (calcarenite). Based on microfauna, Maluf (1975, Fig. 3) correlated the Farmersville Member with the Bear Creek Member. Much of the area between these rock units is covered with alluvium and the details of lithostratigraphy remain in question.

## DESCRIPTION

Previously, trace fossils from the Taylor Group of northeast Texas were described systematically by Richardson (1972, 1975) and Dawson (1976). In the descriptions that follow ichnofossils are treated as biogenic sedimentary structures, and their stratigraphic position and orientation are compared with those of the physical sedimentary features. Biogenic structures are described using the stratinomic (preservational) terminology of Chamberlain (1971, Fig.3), and descriptions of Häntzschel (1975) are followed for established ichnogenera.

### *Farmersville Member*

The Farmersville Member (Brezina, 1974, Fig.1) is exposed in several quarries northwest of Farmersville in Collin County. It has an areal extent of about 1.5 km and an average thickness of 9 m. This lenticular rock body consists of sharply-defined, laterally continuous, thin beds (5–10 cm) composed of alternating quartzose-glauconitic calcarenite and montmorillonitic claystone. Even



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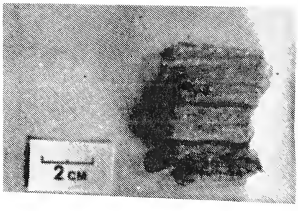
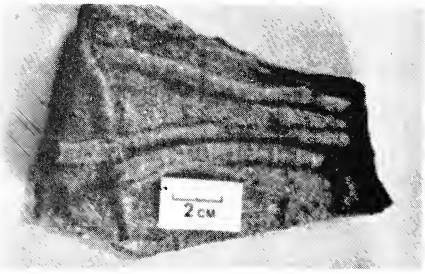



b

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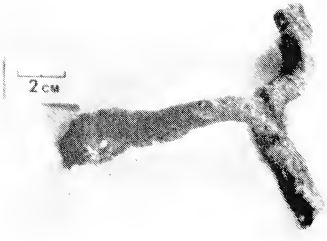
d

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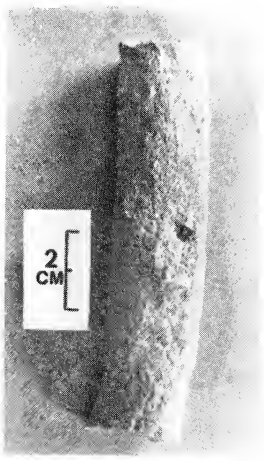



g

Figure 2. a) Quarried slab from Farmersville Member composed of a "mat" of interwoven thalassinoidean burrows. MS C-3X. Scale in cm. b) *Rhizocorallium* sp., fragment of a vertically protrusive horizontal structure. Unit 5, MS C-2. c) *Stipsellus* sp., MS C-2X. d) *Stipsellus* sp., MS C-3X. e) *Thalassinoides* sp., Unit 3, MS C-3. Scale in cm. f) *Thalassinoides* sp., MS C-2X. g) Smooth-walled vertical tube. Unit 5, MS C-2.

parallel laminations (1-2 mm) are the dominant internal sedimentary structure. These well-developed sedimentary features are unique to the Farmersville Member; generally the rock units of the Taylor Group are internally structureless.

Bioturbation is characteristic of the Farmersville Member (Rouse, 1944; Brown, 1949; Brezina, 1974). Trace fossils occur in most of the well-cemented calcarenites but remain undetected, if present, in the interbedded claystones. Biogenic reworking of the calcarenites is commonly so dense that it results in a rock composed of a "mat" of interwoven burrows (Fig. 2a).

Although the Farmersville Member has generally been described as "densely-burrowed," detailed examination reveals that the degree of bioturbation is highly variable. Some beds are completely homogenized with all evidence of primary sedimentary features obliterated; in others, burrows and delicate laminations occur together. These variations occur vertically and also within single beds along an exposure.

Most trace fossils within the Farmersville Member are rather nondescript smooth-walled forms: intergenic and endogenic full-relief burrows. These burrow systems consist of interwoven horizontal cylindrical tubes (5-8 mm in diameter) that can generally be traced for several m across bedding surfaces. Burrow fillings consist of inoceramid prisms, foraminifers, and fine quartz sand cemented with sparry calcite. These traces commonly have an external rim of glauconite.

Recognizable ichnogenera in the Farmersville Member include horizontal, vertically-protrusive *Rhizocorallium* (Fig. 2b), *Stipsellus* (Fig. 2c,d), and *Thalas-*

*sinoides* (Figs. 2e,f). Thalassinoidean structures in the Farmersville Member consist of simple horizontal tubes with Y-bifurcations. These structures lack many features associated with typical *Thalassinoides* sp. (Häntzschel, 1975): longitudinal ridges, vertical shafts, swellings at branching points, and bulbous "dead-end" burrow terminations.

*Rhizocorallium, Stipsellus,* and *Thalassinoides* occur within laminated calcarenites throughout most of the Farmersville Member. However, the upper part of the member contains only vertical burrows (Fig. 2g), which are associated with bimodal cross-laminations.

*Bear Creek Member*

The Bear Creek Member consists of about 9 m of "massive" quartzose calcarenite composed primarily of inoceramid prisms and foraminifers with only minor amounts of glauconite and collophane. In contrast to the Farmersville Member, the Bear Creek Member is intensely bioturbated throughout, and completely homogenized, leaving no evidence of primary depositional features.

The trace fossil assemblage includes relatively large and robust *Thalassinoides* systems with both horizontal and vertical components (Fig. 3, a, b, c, d, e); vertically-protrusive *Rhizocorallium* oriented perpendicular to bedding (Fig. 3f); *Gyrolithes* (?); and numerous vertical tubes with "scratch marks" (Fig. 3g). These forms occur together through the total thickness of the member. The calcarenite burrow fillings are better sorted and contain more sparry calcite cement than the surrounding sediment. Hence, the networks weather into resistant fragments. Also, phosphate nodules (Fig. 3d) are concentrated in thalassinoidean structures.

*Pseudobilobites* sp. (Fig. 3h) is abundant in the subjacent Rockwall Member (Maluf, 1975) and in the upper part of the Wolfe City Formation throughout northeast Texas (Richardson, 1972). However, this ichnogenus is scarce in the Bear Creek Member.

*Rhizocorallium* (Fig. 3f) is similar to U-shaped, spreiten-bearing, vertical burrows described as *Diplocraterion* by Fürsich (1974). However, the forms in the Bear Creek Member display external striae, a significant feature of many U-shaped burrows that was not discussed by Fürsich (1974). According to Häntzschel (1975), external striae (scratch marks) are characteristic of *Rhizocorallium*; the terminology of Häntzschel is retained herein.

## DISCUSSION AND INTERPRETATION

Trace fossils are difficult to classify phylogenetically because: 1) organisms responsible for the structures are rarely preserved; 2) a single animal is capable of producing a variety of traces; and 3) animals which differ taxonomically may create similar traces. Therefore, trace fossils should be considered independently of their producers (Häntzschel, 1975). Even though the biologic affinities of

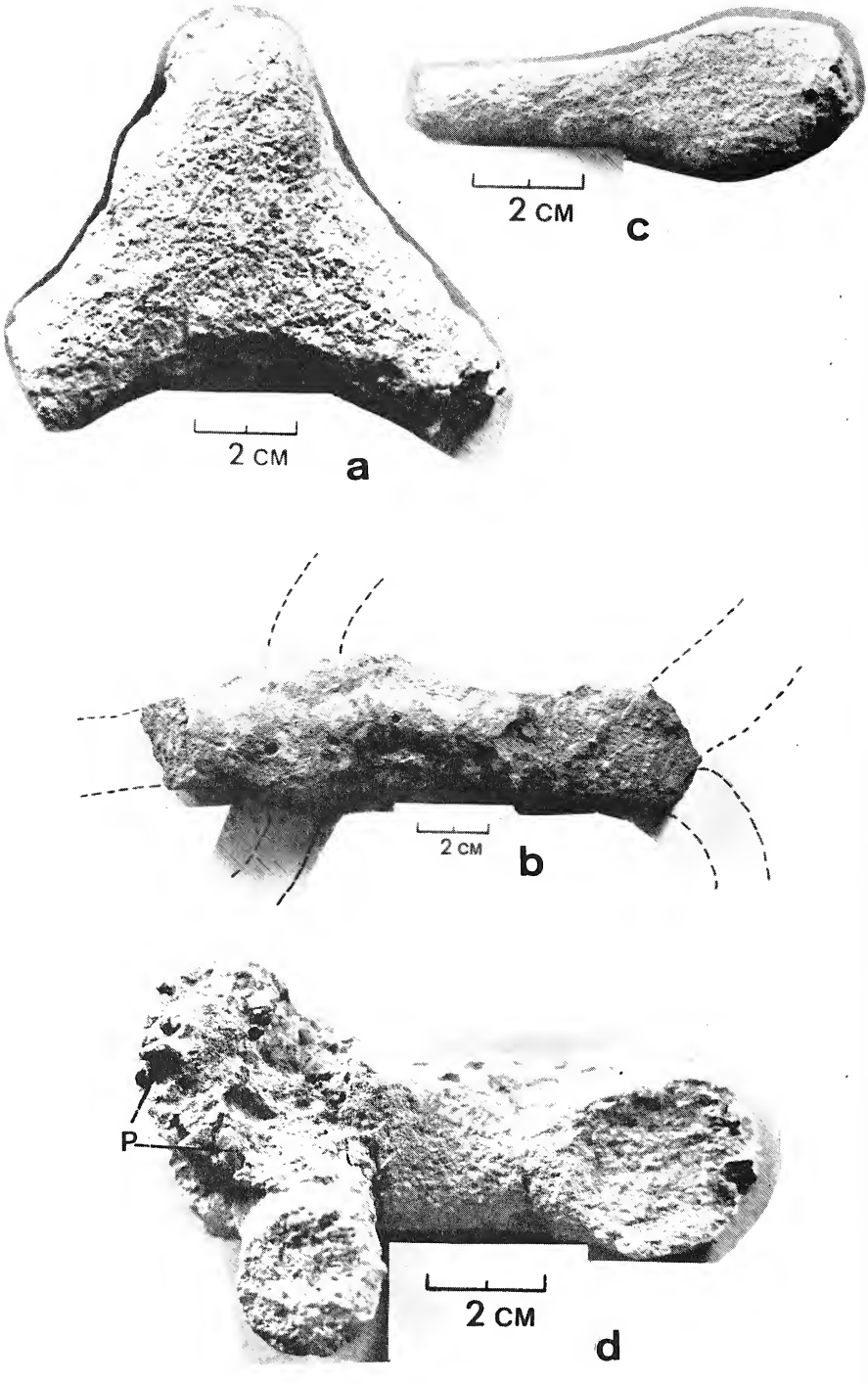
most ichnofossils remain in question, they are potentially valuable in environmental reconstructions.

Creatures dwelling in the sediment interact with the sedimentation processes of the environment. Seilacher (1964, 1967) suggested that these processes largely control the behavior of an animal which in turn controls the morphology of its traces. The underlying assumption of Seilacher's classification is that animals with similar life habits produce similar traces, regardless of their anatomical features. This concept has been used to recognize trace fossil assemblages and explain their distribution by variations in bathymetry. The associations illustrated by Seilacher (1967) show a gradation from vertical (protective) burrows in the shallowest marine environments to highly patterned, horizontal (feeding) burrow systems in deep areas: these traces are said to reflect a change from suspension- to sediment-eating habit.

Seilacher's model predicts that the lateral and vertical distribution of trace fossils in ancient sediments should reflect a bathymetric sequence on a submarine slope. However, later workers have demonstrated that factors other than depth also influence the distribution of trace fossils. In shallow marine environments the complexities of sedimentation can result in local energy conditions or a substrate that is generally associated with another depth zone (Crimes, 1970; Frey, 1971; Hill, 1976). Thus, trace fossils can occur locally in sediment deposited outside the normal depth limits of that assemblage. Using these concepts an interpretation of the Pecan Gap trace fossils will now be undertaken.

The ichnocoenoses of the Pecan Gap Formation in the study area belong to the shallow marine (*Cruziana*) trace fossil assemblage of Seilacher (1964, 1967). The physical and biogenic sedimentary structures of the Farmersville Member are similar to many features that occur in modern and ancient inner shelf deposits (Howard, 1972; Goldring and Bridges, 1973; Cotter, 1975; and Kumar and Sanders, 1976). The dominant primary structure in each case is even parallel laminations. According to Reineck and Singh (1972) laminated shallow marine sands are commonly produced by a process of "settling-out" from suspension following storm-generated turbulence. Cumulative-frequency grain size curves presented by Dawson (1976) are similar to curves illustrated by Visher (1969) and Kumar and Sanders (1976) for Holocene sands deposited from suspension.

Stratigraphic relations indicate that the Farmersville sediments accumulated in a trough shoreward of a submarine high developed in the Ozan Formation. This positive feature acted as a velocity barrier to storm currents (Dawson, 1976). During fair-weather periods, normal suspension clays and silts were deposited, and the storm-generated sands were reworked by burrowing organisms. Longer interstorm periods permitted more thorough churning of the sediment. When the biogenic (disruptive) processes were interrupted by storm activity, previously-deposited storm layers remained only partly reworked as the surviving organisms "escaped" upward to colonize the new sediment horizon. The irregular alternations in sedimentation rate explain the variable degree of bioturbation observed within the Farmersville Member.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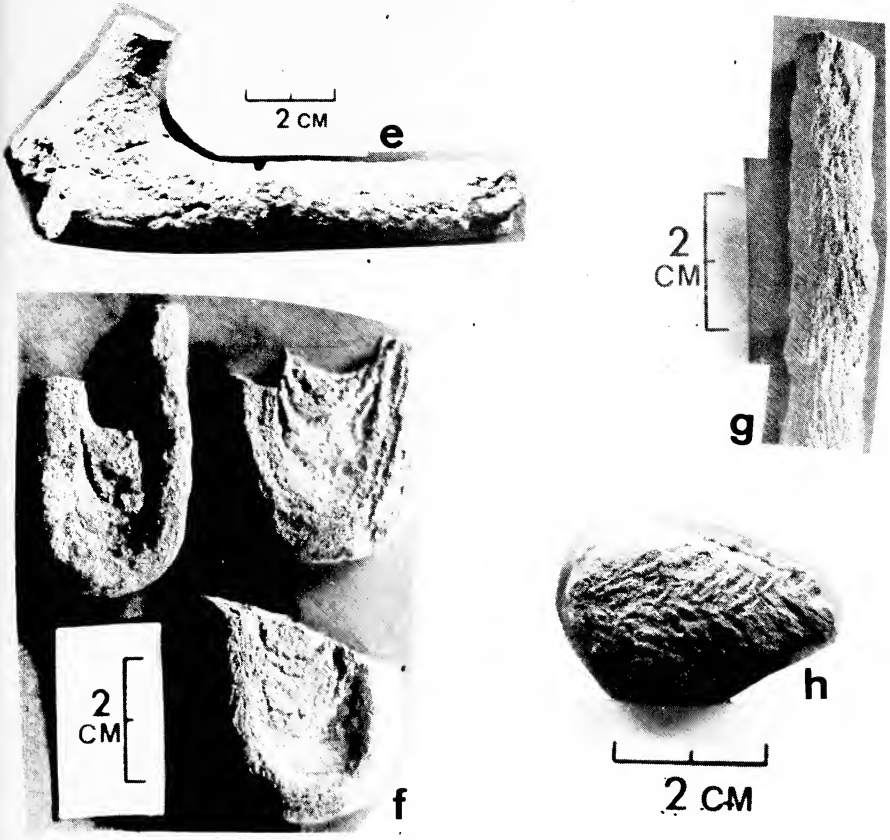


Figure 3. a) *Thalassinoides* sp., note swollen node at branching point. b) *Thalassinoides* paradoxica, MS R-5X. c)*Thalassinoides* sp., "dead-end" burrow termination. d) *Thalassinoides* sp., showing horizontal and vertical components. Note phosphate pebbles (p). e) *Thalassinoides* sp., MS R-5X. f) *Rhizocorallium* sp., Unit 1, MS R-5. g) Vertical burrow displaying "scratch marks." h) *Pseudobilobites* sp., Unit 3, MS R-5.

Ager and Wallace (1970) described an association of horizontal *Rhizocorallium* and small "atypical" *Thalassinoides* from a protected area shoreward of a shallow marine barrier: a situation similar to the trace fossil assemblage of the Farmersville Member. The density of *Stipsellus* and *Thalassinoides* suggests an intensive feeding behavior in an area of low turbulence and generally slow sedimentation rates (Frey, 1971). The textural immaturity of the Farmersville sediments and the abundance of glauconite also support a low energy depositional environment (Dawson, 1976).

The upper part of the Farmersville Member displays bimodal cross-laminations and vertical burrows suggestive of a tidally-dominated zone. Apparently,

as deposition progressed, the Farmersville sediments built upward into a level of higher energy and more rapid sedimentation.

The Bear Creek Member is internally structureless making environmental interpretations relatively difficult. Extensive reworking of these carbonate sands suggests that generally slow and continuous deposition prevailed. The trace fossil assemblage of the Bear Creek Member is similar to crustacean communities found in lower shoreface environments (Howard, 1972). These interpretations are consistent with those proposed by Richardson (1975) for the Wolfe City Formation.

## CONCLUSIONS

Trace fossils are abundant in the Pecan Gap Formation of northeast Texas and because of their autochthonous nature, they provide the most reliable source of paleoenvironmental information. The combined sedimentologic and paleontologic data suggest that Pecan Gap sediments in the study area were deposited on the inner shelf. Farmersville sediments accumulated shoreward of a submarine barrier in an area influenced by storm and tidal currents. Bear Creek sedimentation occurred in the subtidal environment.

Strata of the Taylor Group in northeast Texas are perhaps most similar to Holocene nearshore deposits off the Gulf and Atlantic coasts described by Shepard and Moore (1955) and Howard and Reineck (1972). These sediments occur on a tide-dominated shelf where normal physical and biologic processes are periodically interrupted by storm activity.

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# PRELIMINARY FIELD STUDY OF THE FRACTURE PATTERNS ASSOCIATED WITH THE BALCONES FAULT ZONE IN NORTH CENTRAL TEXAS

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ABSTRACT

A study of the fracture patterns associated with the Balcones Fault Zone in north central Texas has been conducted. The study has revealed the following information: (1) Two general types of fractures are seen in the field; those that cut through strata disregarding lithologic boundaries, and those that are confined to individual beds; (2) well-developed fracture patterns are not confined to limestone sections only; they are also well-developed in the shales; (3) fracture density increases from a few fractures per tens of yards to many fractures per yard in the proximity of major faults, thus providing a reconnaissance tool in field studies; (4) the NE fracture trend of the Balcones Fault Zone is dominant in the carbonate sections. Most shales reflect this trend, but also show a well-developed and sometimes dominant NW fracture trend. Many of these NW trending fractures appear to be genetically unrelated to the Balcones faulting episode.

## INTRODUCTION

The purpose of this study is to investigate the attitude of the fracture patterns in the vicinity of the Balcones Fault Zone in north central Texas. This knowledge will prove advantageous in future engineering geology, structural and tectonic studies, and in our understanding of the tectonic history and structural evolution of the area.

## GEOLOGIC SETTING

### *Location and Previous Work*

The study area is centered in the Waco urban region and encompasses McLennan County, Texas and its immediate vicinity (Fig. 1).

Previous works discussing Balcones faulting are numerous. Those that proved to be most useful in this study include that of Hill (1901), Foley (1926), Link (1929), Melton (1934), Weeks (1945), O. T. Hayward (1957), Burket (1965),

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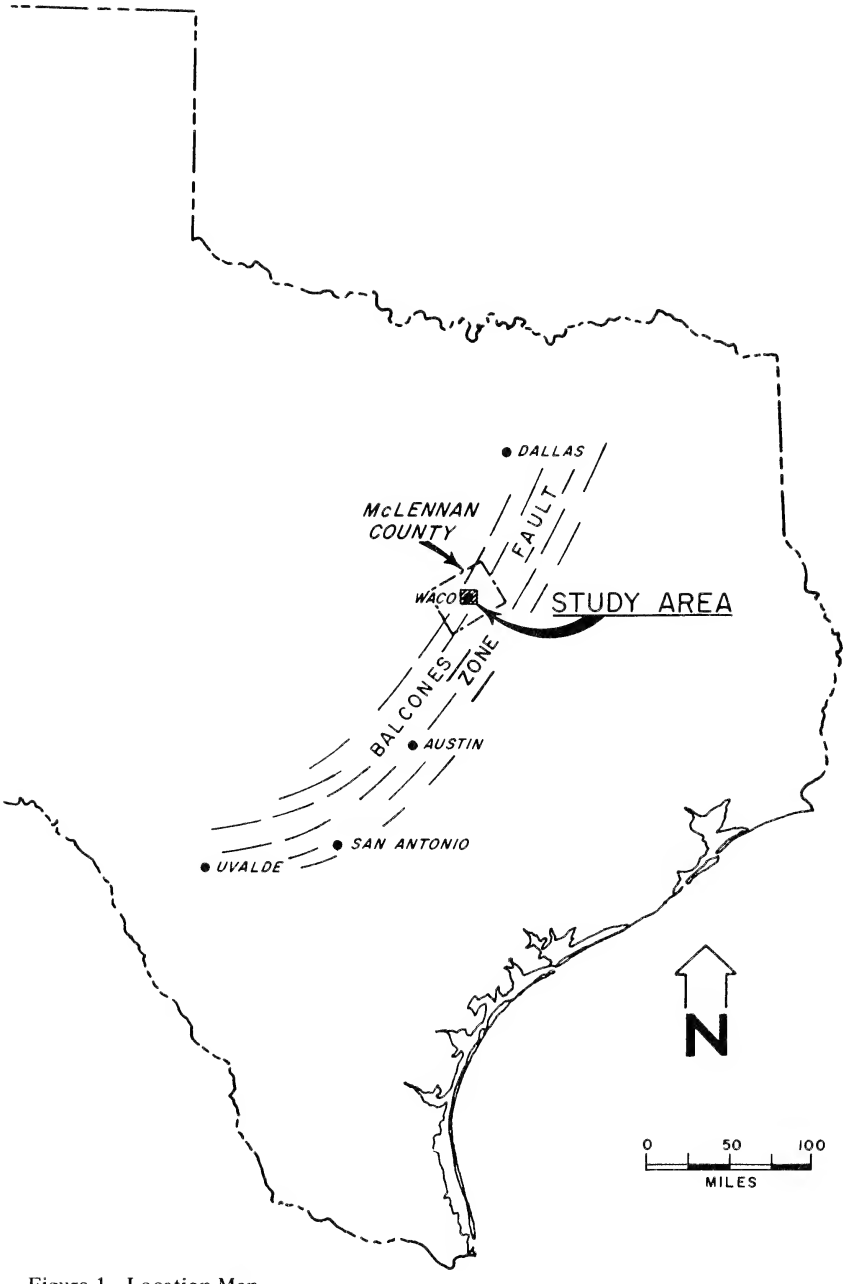


Figure 1. Location Map.

Goodson (1965), Font (1969), Hudson (1972), Font (1973, 1976), and C. Hayward (1976).

*Structure and Stratigraphy*

McLennan County is underlain by eastward dipping beds of the Gulf Coastal Plain. The typical homoclinal structure of the plain is disrupted in the Waco area by the Balcones Fault Zone, a zone of normal faults which extends from west of Uvalde to the Dallas area (Fig. 1). Major faults in the area trend north-northeast and exhibit vertical displacements up to 300 ft. The faults are commonly downthrown to the east, although some are downthrown to the west. Precise dating of the faulting in McLennan County is impossible but there is no evidence of displacement along any of the faults since the Middle Pleistocene (Burket, 1965). The complexity of fault and fracture patterns in the study area as seen from high altitude aerial photographs is shown in Fig. 2. The stratigraphic relationships, depositional history and maximum thickness of the Cretaceous strata in the region of investigation are summarized in Table 1.

## SELECTED REVIEW OF PROPOSED MECHANISMS FOR BALCONES FAULTING

The Balcones Fault System was named by R. T. Hill. Hill (1901) suggested that the faulting probably resulted from adjustments of the Cretaceous strata to the sope of the pre-Cretaceous surface. Foley (1926) concluded that the faulting resulted due to tensional stresses created by subsidence in the East Texas Basin. Link (1929) proposed that faulting resulted from tensional stresses caused by differential settlement of sediments over and around pre-Cretaceous topographic features. Weeks (1945) separated the Balcones, Mexia, and Luling fault zones into 3 separate fracture systems. O. T. Hayward (1957) proposed that Balcones faulting resulted from the inclination of a deep salt "glide plane" causing Gulf Coast Mesozoic and Cenozoic rocks to be fractured over the Ouachita foldbelt by normal gravitational forces. He postulated that the Balcones faults might not penetrate the pre-Cretaceous surface. Goodson (1965) believed that the Ouachita foldbelt was responsible for the Balcones faulting, but never stated if the foldbelt was active or passive in this respect. Hudson (1972) described the complexity of aerial photo alinements in north central Texas and concluded that fracturing resulted from the action of "multiple geologic processes" throughout Cretaceous and early Tertiary time. C. Hayward (1976) has shown that tectonic activity in the area decreased continuously from earliest Cretaceous to pre-Georgetown deposition. Beginning with Georgetown deposition, tectonic activity increased steadily through Austin time. Hayward (1976) has also suggested that the concept of a Cretaceous hinge line may be in error, since deposition apparently prograded into the East Texas Basin.

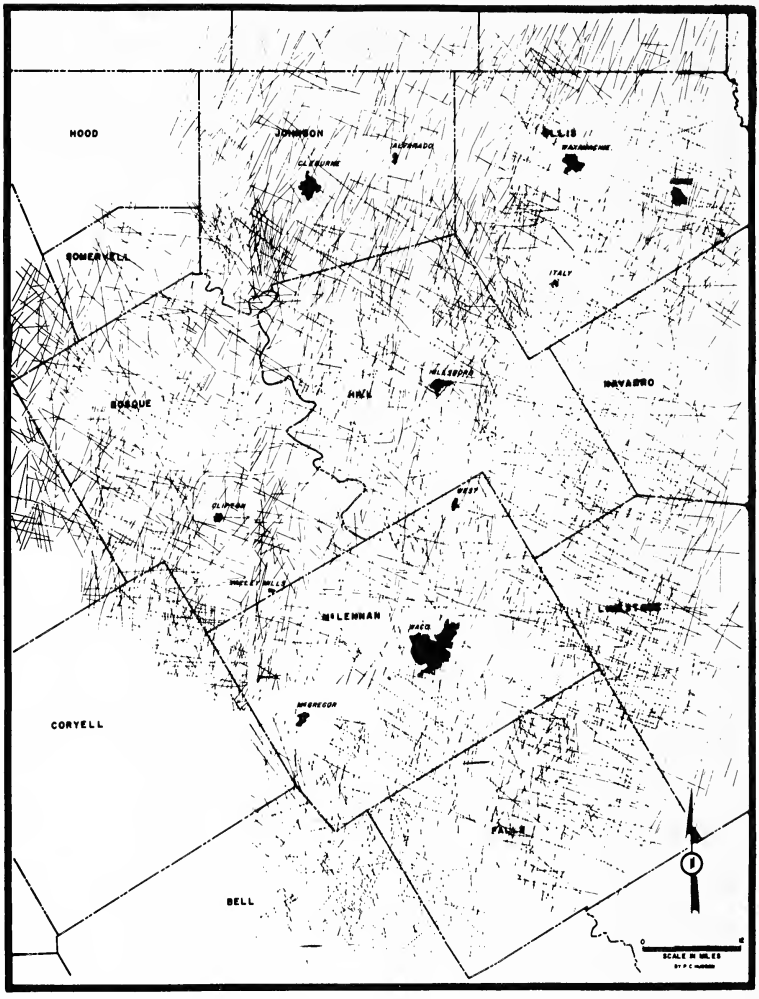


Figure 2. Alinements as seen on high altitude aerial photographs. (after Hudson, 1972).

## FIELD ANALYSIS OF FRACTURE PATTERNS AND CONCLUSIONS

In 1973, Font initiated a systematic study of fracture patterns in some of the shale sections in the Waco area. He recognized 6 prominent fracture trends in the Del Rio Clay (Font, 1973, 1976). Field investigations by the authors of this study have led to the following conclusions regarding fracture patterns in McLennan County:

1. Fractures that cut through lithologic boundaries are related to the Balcones faulting episode. Some of the fractures confined to individual beds are undoubtedly younger and unrelated to the faulting episode. Some may be the result of residual stresses or of swelling and heaving of the clay-shales.

TABLE 1

Stratigraphic Relationship, Depositional History and Maximum Thickness of the Cretaceous Strata in the Local Area.

| Series | Group | Formation and Symbol | Depositional History | Maximum Local Thickness in feet | Maximum Probable Thickness at one time in feet |
|---|---|---|---|---|---|
| Gulf | Taylor | Taylor Marl $K_{ta}$ | Deposited during marine transgressions and regressions. | 250 | 1170 |
| " | Austin | Austin Chalk $K_{au}$ | Deposited during marine transgression with possible fluctuations of the strandline Unconformably underlies the $K_{ta}$. | 250 | 295 |
| " | Eagle Ford | South Bosque Shale $K_{sb}$ | Deposited in a neritic marine environment with poor circulation. Unconformably underlies the $K_{au}$. | 160 | 160 |
| " | " | Lake Waco Fm. $K_{lw}$ | Deposited in a lagoonal environment. Conformably underlies the $K_{sb}$. | 80 | 145 |
| " | Woodbine | Pepper Shale $K_{pe}$ | Deposited in brackish environment. Unconformably underlies the $K_{lw}$. | 70 | 100 |
| Comanche | Washita | Buda Limestone $K_{bu}$ | Nearshore marine deposit. Almost completely eroded locally prior to deposition of $K_{pe}$. | 2 | 35 |
| " | " | Del Rio Clay $K_{dr}$ | Marine regression and transgression. Restricted environment. Conformably underlies the $K_{bu}$. | 85 | 85 |
| " | " | Georgetown Limestone $K_{ge}$ | Shallow marine deposit. | 210 | 210 |

2. Locally well-developed fracture patterns in shales suggest brittle behavior during deformation. This may be related to high strain rates or to a high degree of consolidation before the fracturing took place.
3. Locally well-developed fractures (antithetic, synthetic, feather, etc.) in the vicinity of faults indicate sense of shear and active and passive members. Some locally dense fractures are undoubtedly a result of local stress concentrations related to the curvature of fault planes.
4. Fracture density increases drastically in the proximity of major faults. Fracture spacing can increase from a few fractures per tens of yards to many fractures per yard. This increase in fracture density can be used successfully as a reconnaissance tool in field studies.
5. The NE trend of the Balcones Fault Zone is dominant in limestone sections. The NE trend is reflected in all shales, but a well-developed and sometimes dominant NW trend is recorded in these (Figs. 3-8). The prominent NW fracture trend exhibited by the shale sections appears to be unrelated to the Balcones faulting episode. A great number of the NW trending fractures are short and abut against longer, more prominent NE trending fractures. This indicates that the NW trending fractures are younger than the NE set. In cross-section,

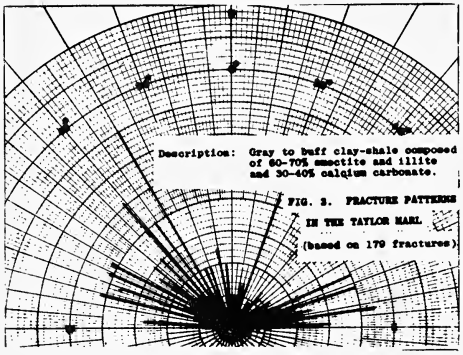


Figure 3. Fracture patterns in the Taylor Marl (based on 179 fractures).

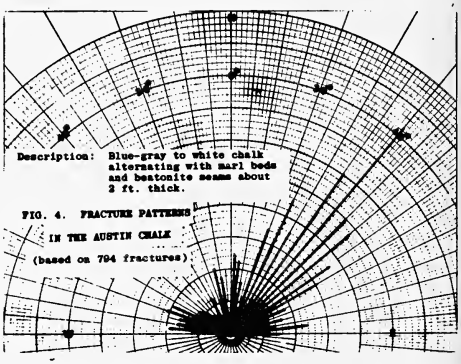


Figure 4. Fracture patterns in the Austin Chalk (based on 794 fractures).

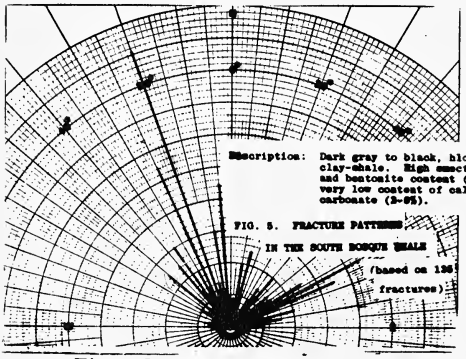


Figure 5. Fracture patterns in the South Bosque Shale (based on 135 fractures).

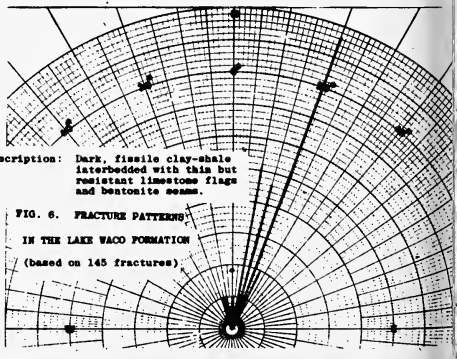


Figure 6. Fracture patterns in the Lake Waco Formation (based on 145 fractures).

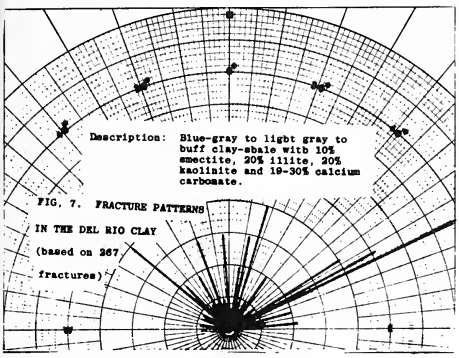


Figure 7. Fracture patterns in the Del Rio Clay (based on 267 fractures).

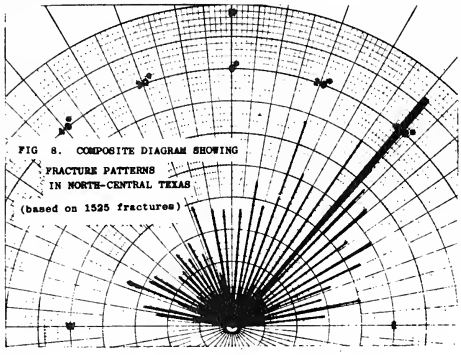


Figure 8. Composite diagram showing fracture patterns in north central Texas (based on 1525 fractures).

NE trending fractures extend through large portions of the shale sections and appear to be closely related to local faults. In contrast, the NW trending fractures are largely confined to individual beds and do not penetrate deeply into the shale sections. These observations lead to the conclusion that most of the NW trending fractures are probably a result of local stresses generated by the swelling and heaving and subsequent contraction of the bentonitic, smectite-rich clay shales.

6. The complexity of the fracture patterns and their modification across major lithologic boundaries indicate the necessity of further, more detailed studies.
7. Lack of outcrops of Pepper Shale and Buda Limestone have not allowed the mapping of sufficient fractures to make any concrete statements. As for the Georgetown Limestone, practically no fractures are recognized in this unit in the study area.

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# LATE PLEISTOCENE POLLEN AND SEDIMENTS: AN ANALYSIS OF A CENTRAL CALIFORNIA LOCALITY

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ABSTRACT

Palynological and sedimentary analyses were conducted on samples obtained from late Pleistocene bog sediments and contiguous-superimposing glacio-fluvial silt loams. These sediments were exposed during gravel quarrying operations just east of Sacramento, California, at the Teichert site. The bog sediments may represent the remnant of an oxbow lake of an ancient (abandoned) channel of the American River.

Uranium and actinium series dates from faunal remains in the overlying and surrounding sediments indicate these deposits are about 100,000 years in age (upper Riverbank Formation).

Reconstructions from the pollen record, faunal remains, and macroscopic plant fragments suggest local bog and oak-riparian vegetation and regional pine parkland vegetation. Climate was apparently cooler than present. This trend is in accordance with a proposed cold climatic period occurring about this time, and these deposits probably indicate correspondence with the Mono Basin glaciation of the Sierra Nevada. Stratigraphic data tend to support the hypothesis. The surrounding and overlying silt-loam layers of the upper Riverbank Formation were apparently deposited during late glacial times by glacial outwash.

Comparisons with modern surface and near-surface pollen from related locations in the Valley and adjoining Sierra indicate a lowering of present vegetation belts along the western Sierra flank 100,000 ya by about 600 to 750 meters.

## INTRODUCTION

As an adjunct to recent radiometric dating of Quaternary soils and sediments (Hansen and Begg, 1970), the opportunity was presented to interpret paleoenvironments by palynological analysis of bog sediments found near Sacramento, in central California. This investigation focused on a late Pleistocene bog apparently formed in an oxbow lake of an ancestral channel of the American River of Riverbank Formation age (e.g. Shlemon, 1967).

The bog and adjacent sediments, exposed during gravel quarrying operations, have been called the Teichert site by Hansen and Begg (1970). The bog is about 10 kilometers (6.2 mi) east of central Sacramento, California (Fig 1).

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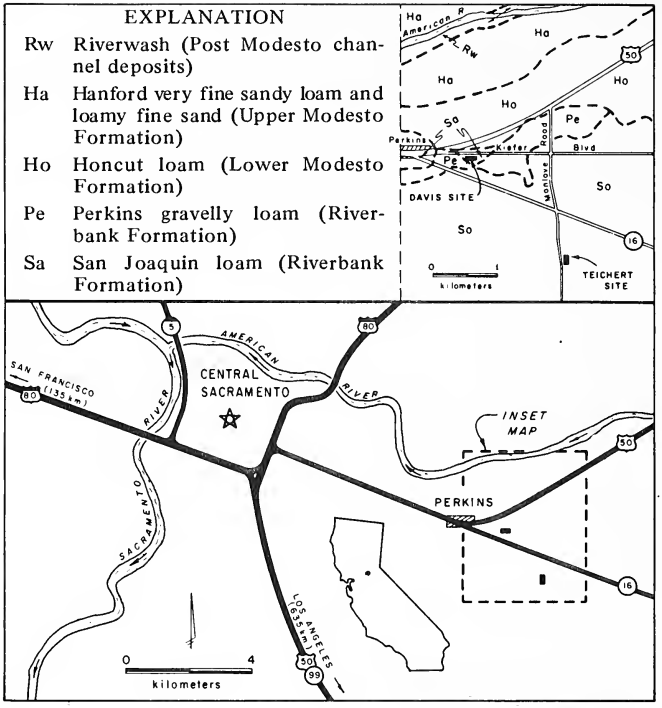


Figure 1. Map of study area east of Sacramento, California. Inset map shows more precise site location and local geology (after Hansen and Begg, 1970).

*Sampling Mechanics*

A series of samples was secured for analysis from the exposed cut. Collecting procedures followed those suggested by Mehringer (1967). In short, the wall was cleaned back about 30 cm, then sample locations were marked with a nail and flagging. Samples of about 200–300 grams each were removed by trowel in peds from bottom to top. Samples were placed in Nasco Whirl-Pak sterilized plastic bags. Subsequently each sample was thoroughly mixed before extraction.

Pollen was sampled from 2 loci in the bog sediments: "A" near the central portion, and "B" near the apparent northern edge (Fig 2). Three samples also were collected from the overlying silt loams, but apparently oxidation in the alkaline sediments destroyed the pollen. In contrast, the bog samples were rich in pollen, wood and other organic material.

The sampling was designed to provide a general overview of the local and regional vegetation (and possibly associated climate) of this central California locality during a specified time range of the late Pleistocene. Samples were taken from the relatively thin and narrow black stratum to (1) acquire pollen spectra of the early, middle and late "bog" center deposits and (2) to deter-

mine intra-bog differences between peripheral and center bog pollen. We hoped that these samples would provide a handle on the relative ranges of variance in the deposit pollen. Our aim was not to determine major climatic or vegetational changes. Our sampling is viewed as only a preliminary step toward better defining our working hypotheses on late Pleistocene vegetation in this region; to enable us to ask more explicit and informative questions.

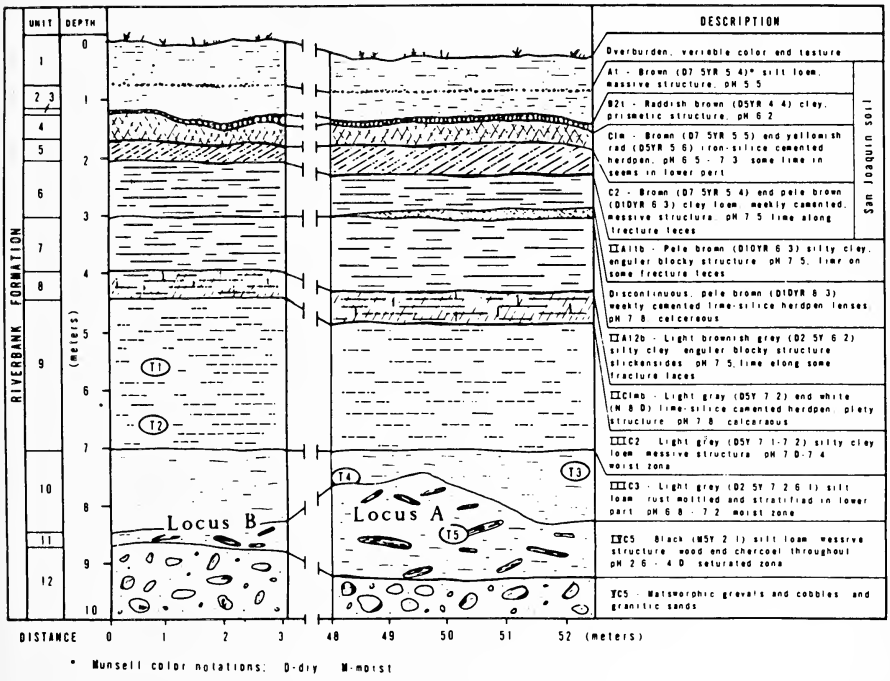


Figure 2. Stratigraphic section of the Teichert Site showing location of pollen samples and radiometrically dated vertebrate fossils and wood (after Hansen and Begg, 1970).

*Laboratory Mechanics*

In following Mehringer (1967), 6 basic steps are required to isolate the fossil pollen from the sediment matrix: (1) HCL swirl for removal of carbonates; (2) soaking and boiling in HF to remove silicates; (3) $HNO_3$ treatment to oxidize organic colloids; (4) acetolysis to remove cellulose; (5) NaOH boiling to destroy humates; and (6) staining the residue with Sulfannin 0 and mounting on a slide with glycerine. Two hundred pollen grains were counted for each sample. We concur with Martin (1963) who indicates this is a significant number statistically since he found only a difference of 4% when compared with slides counted to 2000 grains.

*Stratigraphy of the Teichert Site*

The upper 2 meters consist of modern overburden of variable color and texture and the San Joaquin soil, a brown silty loam with an iron-silica cemented hardpan (A1, B2t, C1m and C2 of Fig 2). Pale brown and light brownish gray silty clays intermingled with 2 discontinuous lime-silica cemented hardpans extend some 2.5 m below this soil (IIA11b, IIA12b, and IIClmb of Fig 2). The next lower 3 to 4 meters include light gray silty clay loam underlain by a light gray silt loam (IIIC2; IIIC3 of Fig 2) both of which contain a faunal assemblage of Rancholabrean age (for a detailed list see Hansen and Begg, 1970).

The bog deposit occurs approximately 7.5 to 9.0 m below the surface (IVC5 of Fig 2). Underlying the bog sediments are unsorted pebbles, cobbles and sands, portion of an ancient gravel bar (VC5 of Fig 2), derived from granitic and metamorphic terrain of the higher Sierra. The bog is about 75 m wide, while the length is presently unknown. Macroscopic plant remains from the bog sediments include wood from Douglas fir (*Pseudotsuga menziesii*), cottonwood (*Populus* sp.), sycamore (*Platanus racemosa*) and willow (*Salix* sp.) as well as unidentified leaf impressions and other plant remains.

Leopold (1964) suggests that detailed study of macroscopic plant remains in peat bogs is useful for indicating local flora and floral succession. These macroscopic plant remains suggest a wet, cool riparian location, while the remains of many mammals, a goose, pond turtles, frogs, snakes and fish in the immediately overlying sediments (5.6–7.5 m depth) indicate the location remained wet with much aquatic life and may also have provided a watering place for non-aquatic mammals. Recovery of *Bison, Camelops, Equus,* and *Mammathus*, and possibly several rodent species indicate nearby or later grasslands. *Sylvilagus* sp. and *Odocoileus* sp. appear discrepant but could be accomodated by a lakeshore or riparian woodland zone in the vicinity. The overlying fauna seemingly reflects a continuance of wet conditions, but it also suggests that the proposed pine oak-grass community was replaced by a prairie similar to the historic one, perhaps during the last interglacial (cf. Suggate, 1974).

While we cannot *in toto* rule out the possibility that some macroscopic plant and animal remains and pollen floated in from the high Sierra, we believe such contamination was minimal as inferred from the evidence presented below.

*Dating*

Age determinations have been discussed in some detail by Hansen and Begg (1970). The Rancholabrean fauna such as found in the sediments overlying bog deposits for the most part probably began to die out from 10,000 to 30,000 years ago.

Data of uranium and actinium series nuclides within the fossils from the Teichert site have provided more accurate age calculations. Five uranium series and 3 uranium-actinium series dates were obtained on the fossils (Hansen and Begg, 1970). The average age of these 8 dates is 98,000 ± 12,000 years B.P.

This age is supported by a radiocarbon date of greater than 38,300 years B.P. for wood taken from the bog (Shlemon and Hansen, 1969). This age is also consistent with the 30,000 to 600,000 yr old Rancholabrean faunal assemblage contained within the sediments.

The local geomorphology has been analyzed by Shlemon (1972). The basal gravels at this locality (VC5, Fig 2) are attributed to the upper Riverbank Formation (Shlemon, 1972). These gravels fill a well-defined channel. Shlemon (1972) notes that "There is not disconformity or buried soil separating the channel gravels from the overlying radiometrically dated sediments. Because of this close stratigraphic proximity, inferentially, the gravels were laid down by an ancestral American River, perhaps 110,000 yrs ago, a time of world-wide cooling as deduced from deep sea cores" (Emiliani, 1961; also see Broecker, 1965). The fine-grained sediments are apparently glacial flour derived from late glacial outwash in the Sierra Nevada (Arkley, 1962; Janda, 1963; Wahrhaftig and Birman, 1965; and Shlemon, 1972).

Dalrymple (1964) has dated interglacial basalt underlying Tahoe (Altonian substage of the Wisconsin Stage) glaciation material in the nearby Sierra by K-Ar as about 60,000 to 90,000 yrs B.P. On this evidence it appears the bog deposit antedates the Tahoe glaciation. It may correlate with Sharp and Birman's (1963) Mono Basin (early Wisconsin) glaciation or Birkeland's (1964) equivalent Donner Lake Glaciation (compare Birkeland, *et al.*, 1971). It also may compare with one of the last "interglacial" cooling periods about 100,000 yrs B.P. as discussed by Suggate (1974). Various other workers have found evidence of such "interglacial" cooling trends (cf. Dansgaard, *et al.*, 1972; Emiliani, 1972; Hayes and Perruza, 1972; Kennett and Huddlestun, 1972; McIntyre and Ruddiman, 1972; Richmond, 1972; and Sancetta, *et al.*, 1972).

*Pollen Analysis*

At Locus A (Fig 3--depths are from upper bog contact with stratum IIIC3) there is a shift through time in pine pollen from 9% near the base to 28% near the upper limits, whereas the oak pollen decreases from 47% to 32%. *Nymphaea*, the water lily, increases from 1% in the bottom sample to 16% in the middle sample and back to 1% near the top. Compositae show a decrease from 19% in the lowest sample to 11% in the middle, increasing to 14% near the top of the bog sediments. Graminae (grass), Cyperaceae (sedge), TCT (Taxodiaceae, Cupresaceae, and Tacaceae, probably mostly *Libocedrus*--cedar and perhaps some *Juniperus*--juniper, *Sequoiodendron*--redwood, *Taxus*--yew, and *Cupressus*--cypress), cheno-ams and the other conifers (*Abies*--fir, *Pseudotsuga*--Douglas fir, etc.) and deciduous trees (*Populus*--cottonwood, *Salix*--willow, *Platanus*--sycamore, etc.) remain fairly stable or have insignificant representation.

Douglas fir (*Pseudotsuga menziesii*), although present in fair amounts macroscopically in the bog sediments, is poorly represented in the pollen spectra and has been lumped in the other conifer category (Fig 3). Leopold (1964) indicates such pollen is usually under-represented, which is a possible explanation.

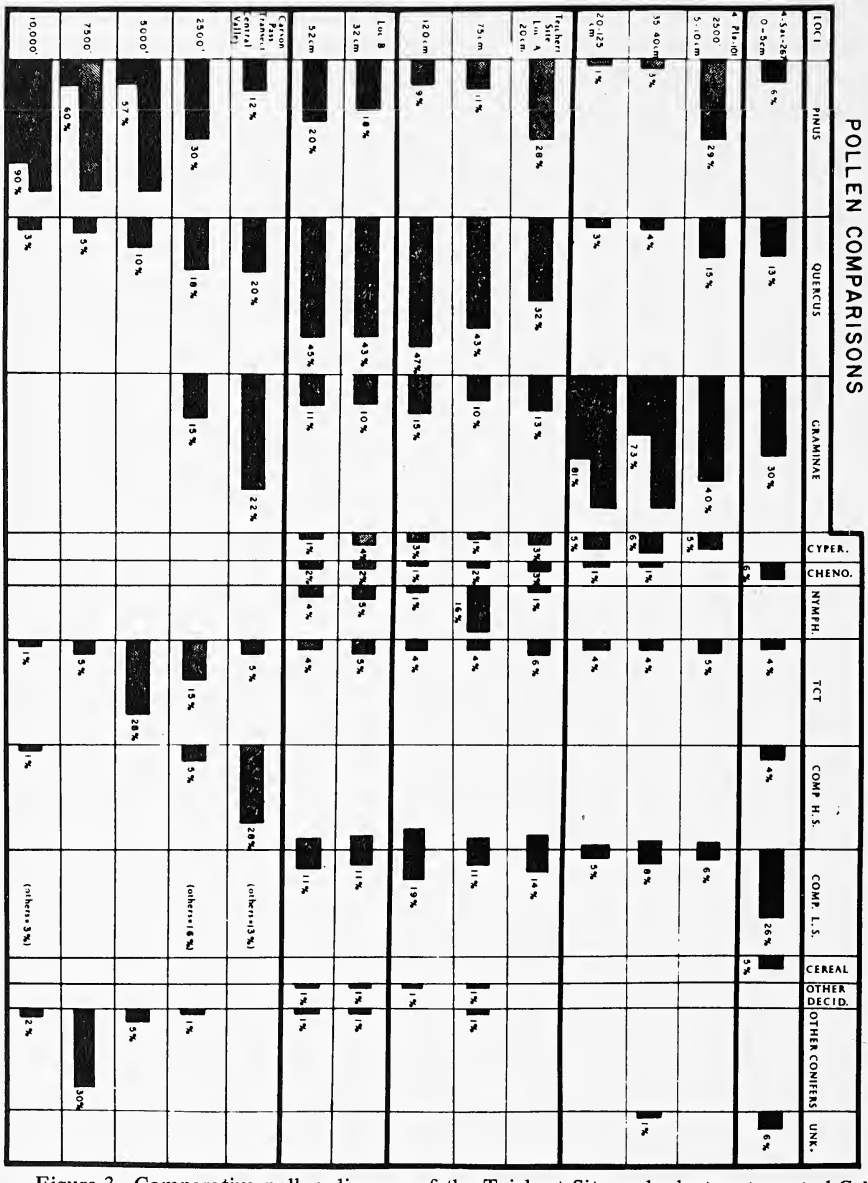


Figure 3. Comparative pollen diagram of the Teichert Site and select east-central California locations.

All the categories in Locus B (Fig 4) show insignificant variation. *Quercus* pollen dominates with 45 and 43% from the lower and upper sample respectively, while *Pinus* is present in 20 and 18% frequency. All others, except *Nymphaea*, are similar in percentile to Locus A.

*Nymphaea* show no significant variation with only 4 and 5% represented at Locus B. The percentile does not approach the 16% of *Nymphaea* found in the

middle sample of Locus A. It is probably that pollen variation between the 2 loci is explainable by intra-bog locational differences and minor variation in pollen dispersal and filtration as discussed by Janssen (1966), Tauber (1967) and others. A possible explanation for some of these differences, based on a study by Maher (1970), is that along the edges of certain lakes vesiculate pollen such as pine accumulates at a greater density than in the center because even light winds can push the floating pollen to the shore. This would account for the higher overall percentages of pine pollen in Locus B (probably bog-lake margin) compared to the lower 2 samples at Locus A. Maher (1970) suggests oxidation-destruction of vesiculate pollen at lake margins is possible under certain conditions (cf. Havinga, 1971). Whether this occurred at Locus B is uncertain, but its potential significance appears minimal owing to the wet anaerobic acidic soil conditions favorable for preservation at the bog locality. The high pine pollen percentage from the upper sample of Locus A may be representative of the penultimate bog environment when free standing water presumably was low, an increase in local or regional pine, or a change in the filtration-dispersion rates.

The higher percentage of *Nymphaea* in the middle of the deposit seemingly is less difficult to explain. Janssen (1966) indicates that most of the lowland herbs are best represented in the local pollen rain. It is noteworthy with respect to later discussion that an over-representation of local pollen will distort the regional pollen rain representation making inferences on climatic (vegetation) changes difficult (Janssen, 1966). Apparently a high number of water lilies were present during the bog climax, about midway in its formation. According to Dapples (1959), during the genesis of bog lakes the false bottom extends for some distance from shore into open water before terminating with a zone of water lilies and similar floating hydrophytes. This false bottom gradually extends lakeward until it covers the pond. Debris then builds up and the pond mat increases until the bog is eventually terminated. Adam (1966), interpreting pollen spectra from Osgood Swamp in the central Sierra Nevada, attributes an increase in *Nymphaea* to cool (Neoglacial) conditions and increased productivity of the lake-swamp. A similar cool period with increased bog productivity at the Teichert site is also a possibility.

Several problems are evident in the palynological analysis of the Teichert site. Is the fossil pollen an adequate measure of regional vegetation and climate? What percent of local, regional and extra-local pollen is present? How much contamination has occurred from fluvial transported pollen?

Potter (1964), Janssen (1966), Tauber (1967), Havinga (1971), and many others mention such basic problems in palynological-environmental reconstruction as (1) amount of pollen production/species, (2) distance of pollen transport, (3) mechanisms of initial and secondary deposition, (4) differential dispersal and filtration of pollen rain, (5) relative preservation of different species in various sediments, and (6) the relation of pollen composition to features of climate, landform, and vegetation. These problems have for the most part been

considered and it is our conclusion that only a general reconstruction of the local and regional vegetation about 100,000 yrs ago is possible.

Havinga (1971) has noted that where wet anaerobic conditions prevail good pollen preservation is guaranteed. Haynes (1964) has indicated that probably the best opportunity for obtaining a *pure* pollen assemblage is furnished by sediments representing semi-permanent or intermittent ponding along the stream channel as inferred at the Teichert site. Bogs themselves have long been used as prime locations for paleoecological reconstruction because of good preservation and relative lack of contamination. If this bog constitutes the remnant of an ancestral oxbow lake, this feature was fairly effectively sealed off from mechanical intrusion of new water, except perhaps during rare extreme flooding. Several small thin lenses of light gray silty loam possibly derived from such flooding were, in fact, noted in the bog sediments, but pollen samples were not collected from these thin sections nor near contacts. Otherwise the bog sediments appear "undisturbed". It must be noted, however, that a single marine foraminifera was identified from the bog sediments by personnel of the Department of Geology, University of California, Davis. It is possible this foraminifera was redeposited from older sediments. Scanning the pollen slides revealed no other foraminifera and this may represent an isolated specimen.

Tauber (1967) has criticized the idea a pollen rain represents the regional pollen production. For instance, in closed deciduous forests pollen carried through the trunk space, above the canopy and in the rain will affect dispersal as well as distance gradients, and pollen carried through the vegetation will be subject to differential filtering. In fact, Tauber (1967) has said"...it is also obvious that various dispersion processes will have a great effect on the deposition of pollen on lake and bog surfaces and that information on these matters is essential for the interpretation of pollen diagrams." Tauber (1967) concludes that in minor lakes and bogs (perhaps like that at the Teichert site), where the trunk-space component is likely to dominate, the area effectively represented will be much smaller than is usually thought. Tsukada (1958) reports that in a forested region most of the pollen precipitation comes from an area within a few kilometers.

It appears the pollen from the Teichert bog is a fair representative of the local late Pleistocene vegetation, that is the bog and surrounding vegetation within several kilometers. The spectra have important implications to late Quaternary events in the Sierra Nevada and Central Valley.

To better interpret the pollen results it is worthwhile to compare the Teichert site pollen with modern pollen spectra obtained from surface sample transects and recent archaeological sites. Such samples are assumed by the researchers to be representative of the local and regional pollen rain/vegetation.

Adam (1967) conducted a pollen transect (Fig 3) from the Central Valley over the Sierra crest at Carson Pass which provides significant comparisons.

Adam's (1967) Central Valley sample (Fig 3) compares closely with that of surface samples from the Blodgett Site (CA-Sac-267) (Fig 3; Germeshau-

sen, 1969). This archaeological midden is located 7 kilometers (4.3 mi) east of the Teichert site on the first terrace above the Consumnes River near Sloughhouse, California. Each spectrum is representative of the modern open grasslands with scattered oak. This is also the present vegetation pattern at the Teichert site locality and extends to the present riparian belt along the American River to the north (Fig 1). As the elevation increases in Adam's transect ascending the Sierra, the *Pinus* pollen significantly rises from 12% in the Valley to 30% at 760 m (2500 ft), to 57% at 1520 m (5000 ft), increases slightly to 60% at 2280 m (7500 ft), and finally to 90% at 3040 m (10,000 ft). *Quercus*, Graminae, and Compositae, on the other hand, correspondingly decrease, with the latter 2 not represented at 1500 m and higher. TCT and other conifers, notably *Abies* (fir), oscillate, with TCT increasing to its highest frequency (28%) at 1500 m before dropping off, and the other conifers peaking at 2280 m with 30%.

Modern pollen rain samples from the Spring Garden Ravine archaeological site (CA-Pla-101) in the Sierra upper foothills (elevation 760 meters or 2500 ft) 60 kilometers (42 mi) east-northeast of the Teichert site (Fig 3) correlate well with the present pine-oak woodland (Matson, 1970). This site adjoins a bog. Subsurface samples radiometrically dated at 3350 ± 110 B.P. (GaK-2246)-still within the present day climatic regime (Medithermal)-suggest vegetation was a lightly wooded oak grassland with or without low pollen-producing chaparral species. Changes in pollen spectra/vegetation from the older pattern to the modern pattern can be attributed to cessation of aboriginal practices of burning (Matson, 1970). The results of the modern pollen rain are thought by Matson to better reflect natural vegetation conditions during the Medithermal.

What is believed diagnostic in comparing results of these other studies with those of the Teichert bog is the similarity at about 750 m of the pine and grass pollen. High *Quercus* values at the Teichert site (compared to the samples obtained from the higher elevation locations) we attribute to local riparian-like growth not present at either Adam's or Matson's test locations. Adam (1967), in fact, notes that neither riparian nor montane-chaparral communities are well represented in his transects.

## INTERPRETATIONS AND EXPLANATIONS

It appears that the local and regional vegetation around the Teichert locality at the time of bog formation, about 100,000 yrs ago, differed from present or historic Valley vegetation, a reflection of glacio-climatic conditions in the adjacent Sierra Nevada. The 98,000 yr old average age of these upper Riverbank Formation sediments corresponds closely to a cold period which occurred about this time (cf. Broecker, 1965). We assume that vegetation zones of the Sierra, as noted by Adam (1967), have retained their integrity and relative positions during vertical movements imposed by Pleistocene climatic changes, that lag was minimal, and that glacial climates depress vegetational belts relative to their

interglacial positions, as do minor climatic fluctuations such as have occurred during the Holocene. Localized conditions such as riparian belts and bogs add complexity to this concept.

The changes within the bog are a result of vegetational responses to evolving bog conditions and perhaps a minor shift in climate (e.g. *Pinus* increases at Locus A). At the end of bog life cooler conditions may have prevailed than during the beginning, but it may also be that during the early stages of bog formation winds moved the vesiculate pine pollen from the center of the oxbow lake (Locus A) to the margin (Locus B). In either case, a higher pine percentage is found in the bog sediments than prevails in surface samples in the area today. The silt loam layers superposing the bog sediments are characteristic of glacial flour, an indication of rapid aggradation of glacio-fluvial outwash and hence a glacial recession with overloading of the ancestral American River. As early as 1885 and 1889, Russell demonstrated that pluvial events in western North America could be linked stratigraphically to local glaciation in the Sierra and elsewhere. The Teichert bog was thus probably formed just prior to deposition of the overlying sediments during a cold episode corresponding to high Sierra glaciation (i.e., Mono Basin) or part thereof.

If the present vegetation in the Sierra Nevada can be extrapolated to the bog, then a lowering of the present vegetation belts by about 600 to 750 meters (2000 to 2500 ft) is postulated for the period of bog formation. Local channel conditions during this period apparently favored a heavy oak stand and local aquatic plants such as sedge and water lily. The aquatic plants decreased as the bog lost its effective hydrophilous attributes and became filled. The riparian vegetation was apparently symbiotic with a surrounding pine parkland. The tree remains of Douglas fir, which presently is found on moist slopes of the west central Sierra below 1500 m (5000 ft) and above 300 m (1000 ft), seemingly add further confirmation.

Martin (1964) utilizing data from 9 pollen spectra thought to be of full glacial age in the American Southwest, notes that to find a modern pollen count similar to the full glacial pollen spectra it is necessary to sample lake sediments or soils 800 to 1200 m (2625 to 3937 ft) higher in elevation than the fossil localities. Furthermore, Martin (1964) indicates the pollen type most likely to reveal the full glacial change is pine. It is believed that these results from the California Central Valley are analogous to those in the Southwest. While this model is still conjectural, empirical evidence is accumulating, and further effort should be made to find correlative or non-correlative data. Along this line it is worth mentioning that a few undated relict soil structures possibly characteristic of cooler areas have been found along the east side of the Central Valley. These as yet unreported features include probable stone nets and stripes. However, Shlemon, Begg and Huntington (1973) have noted that some such structures along the Sacramento Valley edge are structurally controlled.

This study reaffirms the importance of interdisciplinary cooperation needed to reconstruct paleoenvironments.

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# VEGETATION TYPES OF CHAMBERS COUNTY, TEXAS

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ABSTRACT

The vegetation of Chambers County, Texas, is described and mapped on the basis of field reconnaissance. Eight vegetation types are recognized. Moisture availability, salinity, and soil texture are identified as probable determinants of the pattern of vegetation distribution.

## INTRODUCTION

Chambers County, Texas, lies just inland from the Gulf of Mexico on the north and east shores of Galveston Bay, and in many respects, it is typical of the coastal environment of eastern Texas and western Louisiana. Because Chambers County contains a wide range of vegetation—from prairie to forest—it is of interest to describe the vegetation pattern and to attempt to identify the contributing environmental factors.

Documentation of the present distribution of vegetation types seems particularly worthwhile, given that Chambers County is located between two rapidly growing metropolitan centers (Beaumont—Port Arthur and Houston—Galveston), and will undergo change with increasing industrial and residential development. Thus, the data reported here are intended to provide both a baseline for evaluation of the impact of development and a regional overview for more intensive studies of coastal ecosystems.

This work is part of a larger attempt to describe the natural environment of Chambers County and to develop mechanisms facilitating land-use decision-making (Rowe, *et al.*, 1974). Primary emphasis here is description of existing native (i.e., not cultivated) plant assemblages.

Three sources have been used: published descriptions of the vegetation of the Gulf Coast, published information on the effects of environmental factors on vegetation, and personal observation and field reconnaissance. Quantification of vegetation composition was outside the scope of the present study.

### *Site*

Chambers County is a broad, flat, poorly drained plain, of about 221,000 ha with slopes less than 3% and elevation mostly less than 11 m. It is composed of

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Pleistocene and Holocene deltaic and fluviatile deposits (Rowe, *et al.*, 1974; USDA, 1976). The soils are predominantly clays and silt-loams of the Beaumont-Morey-Lake Charles and Morey-Anahuac-Frost Associations. The climate is warm and wet with 1309 mm average annual rainfall and a growing season of approximately 261 days (USDA, 1976).

Chambers County lies entirely within the Gulf Prairie and Marsh Vegetation Area of Texas (Gould, 1969), which is characterized by "level grassland, low flat woodlands, especially near the streams, swamps and . . . marshes" (Correll and Johnston, 1970). The types of vegetation mapped by Küchler (1964) included Bluestem-Sacahuiste Prairie, Oak-Hickory-Pine Forest, Southern Floodplain Forest, and Southern Cordgrass Prairie. Fisher, *et al.*, (1972, 1973) mapped 12 assemblages of rooted vegetation, including 4 types of marsh, a single prairie grassland type, 2 types of swamp, 3 types of woodland, and a successional forest type. The Soil Conservation Service recognized 7 types of prairie and marsh in Chambers County (USDA, 1976); the U.S. Fish and Wildlife Service recognized 8 distinct marsh zones (U. S. Department of Interior, 1952).

These publications, plus the earlier surveys of Tharp (1926, 1939), constitute most of the available information on the vegetation of Chambers County, and they were used as the basis for reinterpretation of the vegetation. A reinterpretation was deemed necessary because none of the above works comprehensively describe the vegetation. Küchler's work (1964) is very general, Fisher, *et al.*, (1972, 1973) erred in assemblage descriptions (see below) and in setting assemblage boundaries (Solomon and Smith, 1973); and the U.S. Government information does not treat all assemblages uniformly.

To compile the new description, we followed Küchler (1964) in dividing the landscape first according to physiognomy (i.e., whether forest, woodland, brushland), and second according to species composition (identity of prominent or predominant species).

Once the landscape had been divided into physiognomic types, each type was subdivided into assemblages, or groups of species commonly found together. Eight assemblages were identified (Table 1): floodplain cypress swamp, floodplain hardwood forest, upland oak-pine forest, streamside woodland, bluestem prairie, cordgrass prairie, fresh marsh, and brackish marsh. They are described in Section II. For convenience, these assemblages are discussed as discrete entities; actually in most cases they appear to intergrade with one another.

Vascular plant species lists for the assemblages (Tables 2-5) were compiled from personal records and from observations in nearby areas (Lohse, *et al.*, 1973). They are not necessarily complete; they should be regarded as preliminary checklists. Plant nomenclature follows Correll and Johnston (1970).

A map (Figure 1) was produced by copying forest, prairie, and marsh boundaries from 1:8,000 SCS aerial photographs flown in 1970, and subdividing forest, prairie, and marsh according to species composition determined by field reconnaissance. Environmental factors which appear to strongly influence assemblage distribution were identified on the basis of field observations.

TABLE 1

Plant Assemblages of Chambers County.

| I. Forest | Major Species |
|---|---|
| Cypress Swamp | bald-cypress, water elm, buttonbush, water hickory |
| Floodplain Hardwood Forest | cedar elm, sugarberry, hawthorn, water oak, sweetgum, green ash |
| Upland Oak-Pine Forest | loblolly pine, sweetgum, willow oak, water oak, southern red oak, magnolia |
| Steamside Woodland | sugarberry, hawthorn, water oak, willow oak |
| **II. Prairie** | **Major Species** |
| Bluestem Prairie | little bluestem, brownseed paspalum, switchgrass, indiangrass, eastern gamagrass |
| Cordgrass Prairie | gulf cordgrass, knotroot bristle grass |
| **III. Marsh** | **Major Species** |
| Fresh Marsh | common rèed, saw grass, cutgrass, big cordgrass, California bulrush |
| Brackish Marsh | marsh-hay cordgrass, seashore salt grass, salt-marsh bulrush, olney bulrush |

TABLE 2

Scientific names of plant species referred to in text. (After Correll and Johnston, 1970, except where authority is cited.)

Alligatorweed – *Alternanthera philoxeroides*
American beautyberry – *Callicarpa americana*
Ash, green – *Fraxinus pensylvanica*
Bald-cypress – *Taxodium distichum*
Bluestem – *Andropogon* spp.
Bluestem, Big – *A. gerardi*
Bluestem, Little – *Schizachyrium scoparium*
Bluestem, Silver – *Bothriochloa saccharoides*
Bulrush, California – *Scirpus californicus*
Bulrush, Salt Marsh – *S. maritimus*
Bulrush, Olney – *S. olneyi*
Bushybeard – *Andropogon glomeratus*
Button bush – *Cephalanthus occidentalis*
Carpetgrass – *Axonopus affinis*
Cattail – *Typha latifolia, T. dominguensis*
Chinese Tallow – *Sapium sebiferum*
Common Reed – *Phragmites communis*
Cordgrass, Big – *Spartina cynosuroides*
Cordgrass, Gulf – *S. spartinae*

Table 2 (Continued)

Cordgrass, Marsh-hay – *S. patens*
Cordgrass, Smooth – *S. alterniflora*
Crossvine – *Bignonia capreolata*
Cutgrass – *Zizaniopsis miliacea*
Eastern Gamagrass – *Tripsacum dactyloides*
Elm, Cedar – *Ulmus crassifolia*
Elm, Water – *Planera aquatica*
Greenbriar – *Smilax* spp.
Hawthorn – *Crataegus* spp.
Hickory – *Carya* spp.
Hickory, Water – *C. aquatica*
Huisache – *Acacia farnesiana*
Indiangrass – *Sorghastrum avenaceum*
Knot-root Bristlegrass – *Setaria genicultata*
Longtom – *Paspalum lividum*
Magnolia – *Magnolia grandiflora*
Needlegrass – *Juncus roemerianus*
Oak, Basket – *Quercus prinus*
Oak, Laurel – *Q. laurifolia*
Oak, Live – *Q. virginiana*
Oak, Post – *Q. stellata* or *Q. similis*
Oak, Southern Red – *Q. falcata*
Oak, Water – *Q. nigra*
Oak, Willow – *Q. phellos*
Palmetto – *Sabal minor*
Paspalum, Brownseed – *Paspalum plicatulum*
Paspalum, Seashore – *P. vaginatum*
Poison Ivy – *Rhus toxicodendron*
Pine, Loblolly – *Pinus taeda*
Pine, Longleaf – *P. palustris*
Pine, Shortleaf – *P. echinata*
Rattan – *Berchemia scandens*
Rattle Bush – *Sesbania drummondii*
Sacahuiste – *Spartina spartinae*
Savanna panicum – *Panicum gymnocarpon*
Saw Grass – *Cladium jamaicense*
Sea Myrtle – *Baccharis halimifolia*
Seashore Saltgrass – *Distichlis spicata*
Smutgrass – *Sporobulus indicus*
Sugarberry – *Celtis laevigata*
Sweetgum – *Liquidambar styraciflua*
Switch Grass – *Panicum virgatum*
Sycamore – *Platanus occidentalis*
Trumpet Creeper – *Campsis radicans*
Tupelo – *Nyssa aquatica*
Vasey Grass – *Paspalum urvillei*
Willow – *Salix nigra*
Willow, Water – *Justicia lanceolata*
Woodgrass – *Chasmanthium sessiliflorum, C. laxum*
Yaupon – *Ilex vomitoria*

TABLE 3

Checklist of Forest Species in Chambers County

| Trees | Floodplain Hardwood Forest | Cypress Swamp | Upland Oak-Pine Forest | Streamside Woodlands |
|---|---|---|---|---|
| *Acer negundo* – box elder | x | | | x |
| *A. rubrum* – red maple | x | x | | |
| *Carpinus caroliniana* – ironwood | x | | x | |
| *Carya aquatica* – water hickory | x | x | | |
| *C. cordiformis* – bitternut hickory | | | x | |
| *C. illinoiensis* – pecan | x | | | |
| *C. laciniosa* – shellbark hickory | | | x | |
| *Celtis laevigata* – sugar hackberry | x | | x | x |
| *Crataegus brachyacantha* – blueberry hawthorn | x | | x | |
| *C. spathulata* – littlehip hawthorn | | | | x |
| *C. uniflora* – oneflower hawthorn | | | | x |
| *C. viridis* – green hawthorn | | | | x |
| *Diospyros virginiana* – common persimmon | x | | x | |
| *Fraxinus americana* – white ash | | | x | |
| *F. pensylvanica* - green ash | x | | | x |
| *Gleditsia aquatica* – water honeylocust | x | | | x |
| *G. triacanthos* – common honeylocust | | | x | x |
| *Ilex opaca* – American holly | | | x | |
| *Juglans nigra* – black walnut | | | x | |
| *Juniperus silicicola* – southern red cedar | | | | x |
| *J. virginiana* – eastern red cedar | | | | x |
| *Liquidambar styraciflua* – sweetgum | x | | x | |
| *Magnolia grandiflora* – Southern magnolia | | | x | |
| *Melia azedarach* – chinaberry tree | | | | x |
| *Morus alba* – white mulberry | | | x | |
| *M. rubra* – red mulberry | | | x | |
| *Parkinsonia aculeata* – retama | | | | x |
| *Pinus taeda* – loblolly pine | | | x | |
| *Platanus occidentalis* – American sycamore | x | | | |
| *Populus deltoides* – eastern cottonwood | x | | | |
| *Prunus angustifolia* – chickasaw plum | | | x | |
| *P. mexicana* – Mexican plum | | | x | x |
| *P. serotina* – black cherry | | | x | |
| *P. umbellata* – flatwood plum | | | x | |
| *Quercus falcata* – southern red oak | x | | x | |
| *Q. laurifolia* – laurel oak | x | | x | |
| *Q. lyrata* – overcup oak | x | | | |
| *Q. nigra* – water oak | x | | x | x |
| *Q. phellos* – willow oak | x | | x | x |
| *Q. prinus* – chestnut oak | x | | x | |
| *Q. shumardii* – shumard oak | x | | | |
| *Q. similis* – bottomland post oak | x | | x | |
| *Q. stellata* – post oak | | | x | |
| *Q. virginiana* – live oak | x | | | x |
| *Salix nigra* – black willow | x | | | x |
| *Sapindus saponaria* – western soapberry | | | | x |
| *Taxodium distichum* – bald-cypress | | x | | |
| *Tilia caroliniana* – Carolina basswood | | | x | |
| *Ulmus alata* – winged elm | x | | x | x |
| *U. americana* – American elm | x | | | |
| *U. crassifolia* – cedar elm | x | | | x |
| *U. rubra* – slippery elm | x | | | |
| *Zanthoxylum clava – herculis* – toothache tree | | | | x |

Table 3 (Continued)

| Shrubs | Floodplain Hardwood Forest | Cypress Swamp | Upland Oak-Pine Forest | Streamside Woodlands |
|---|---|---|---|---|
| *Amorpha fruticosa* – bastard indigo | | | | x |
| *Aralia spinosa* – Devil's walking stick | | | x | |
| *Ascyrum hypericoides* – St. Andrew's cross | | | x | x |
| *A. stans* – Atlantic St. Peter's wort | | | x | x |
| *Asimina triloba* – pawpaw | | | x | |
| *Baccharis halimifolia* – sea myrtle | | | | x |
| *Bumelia lanuginosa* – gum bumelia | | | x | x |
| *Callicarpa americana* – American beautyberry | | | x | |
| *Cephalanthus occidentalis* – common buttonbush | | x | | |
| *Chionanthus virginica* – old-man's beard | | | x | |
| *Citrus trifoliata* – bitter orange | x | | x | x |
| *Cornus drummondii* – roughleaf dogwood | | | x | |
| *C. florida* – flowering dogwood | | | x | |
| *Erythrina herbacea* – coral bean | | | x | x |
| *Forestiera acuminata* – swamp privet | x | | | x |
| *F. ligustrina* – privet | | | x | x |
| *Halesia diptera* – silverbell | | | x | |
| *Ilex decidua* – possum-haw | x | | | |
| *I. vomitoria* – yaupon | x | | x | x |
| *Ligustrum quihoui* – wax-leaf ligustrum | | | x | |
| *Malvaviscus arboreus* – turks cap | x | | x | |
| *Myrica cerifera* – wax-myrtle | | | x | |
| *Ptelea trifoliata* – woolly hoptree | | | x | x |
| *Rhamnus caroliniana* – Carolina buckthorn | | | x | x |
| *Rhus copallina* – flameleaf sumac | | | | x |
| *Rivina humilis* – rougeplant | | | x | x |
| *Sabal minor* – dwarf palmetto | x | | x | |
| *Sambucus canadensis* – common elder-berry | x | | | |
| *Sassafras albidum* – sassafras | | | x | |
| *Symphoricarpus orbiculatus* – coral-berry | | | x | |
| *Symplocos tictoria* – horse-sugar | | | x | |
| *Vaccinium arboreum* – farkleberry | | | x | |
| *Viburnum dentatum* – southern arrow-wood | | | x | |
| *V. nudum* – possum-haw | | | x | |
| *V. prunifolium* – black-haw | | | x | |
| *V. rufidulum* – rusty black-haw | | | x | |
| **Vines** | | | | |
| *Ampelopsis arborea* – peppervine | x | | x | x |
| *Aristolochia tomentosa* – Wooly Dutchman's pipe | | | x | |
| *Berchemia scandens* – rattanvine | x | | | x |
| *Bignonia capreolata* – crossvine | | | x | |
| *Campsis radicans* – trumpet creeper | x | | x | |
| *Cissus incisa* – ivy treevine | | | x | |
| *Cocculus carolinus* – snailseed | | | x | |
| *Gelsemium sempervirens* – Carolina jessamine | | | x | x |

Table 3 (Continued)

| Vines (Continued) | Floodplain Hardwood Forest | Cypress Swamp | Upland Oak-Pine Forest | Streamside Woodlands |
|---|---|---|---|---|
| *Lonicera japonica* – Japanese honeysuckle | | | x | x |
| *L. sempervirens* – trumpet honeysuckle | | | x | x |
| *Lygodium japonicum* – Japanese climbing fern | x | | | |
| *Matelea gonocarpa* – angle-pod milkvine | | | x | |
| *Melothria pendula* – meloncito | | | x | |
| *Mikania scandens* – climbing hempweed | | | | x |
| *Parthenocissus quinquefolia* – Virginia creeper | x | | x | |
| *Passiflora incarnata* – maypop | | | | x |
| *P. lutea* – dwarf passion flower | | | x | |
| *Rhus toxicodendron* – poison ivy | x | | x | x |
| *Rubus louisianus* – blackberry | x | | x | x |
| *R. trivialis* – southern dewberry | | | x | x |
| *Smilax bona-nox* – saw greenbriar | | | x | x |
| *S. glauca* – catbriar | | | x | x |
| *S. hispida* – bristly greenbriar | | | x | |
| *S. laurifolia* – blaspheme vine | x | | | |
| *S. pumila* – sarsaparilla vine | | | x | |
| *S. rotundifolia* – common greenbriar | | | x | x |
| *Trachelospermum difforme* – star jasmine | x | | | |
| *Vitis aestivalis* – summer grape | x | | | |
| *V. mustangensis* – mustang grape | x | | x | |
| *V. rotundifolia* – muscadine grape | x | | | |
| **Herbs** | | | | |
| *Argemone albiflora* – white prickly poppy | | | x | |
| *Arisaema triphyllum* – Jack-in-the-pulpit | x | | | |
| *Aster lateriflorus* – starved aster | | | x | |
| *Athryium filix-femina* – Southern ladyfern | | | x | |
| *Bidens bipinnata* – Spanish needles | | | x | |
| *Boehmeria cylindrica* – smallspike false nettle | x | | | |
| *Cassia occidentalis* – coffee senna | | | x | x |
| *Clematis crispa* – blue jasmine | x | | | |
| *Clitoria mariana* – Atlantic pigeonwings | | x | | |
| *Coreopsis cardiminaefolia* – Manzanilla silvestre | | | x | |
| *Crepis pulchra* – showy hawksbeard | | | x | |
| *Cuscuta compacta* – dodder | x | | x | x |
| *Diclipera brachiata* – dicliptera | x | | x | |
| *Desmodium paniculatum* – panicle tick clover | | | x | |
| *Elephantopus carolinianus* – leafy elephantfoot | | | x | |
| *E. nudatus* – naked elephantfoot | | | x | |
| *E. tomentosus* – hairy elephantfoot | | | x | |
| *Eupatorium coelestinum* – mist flower | x | | x | x |
| *E. serotinum* – late flowering eupatorium | | | x | x |
| *Euphorbia bicolor* – Snow-on-the-prairie | | | | x |
| *E. cyathophora* – wild poinsettia | | | x | |
| *Galactia volubilis* downy milkpea | | | x | |
| *Geum canadense* – white avens | | | x | x |
| *Habenaria clavellata* – white fringe orchid | | | x | |
| *Hydrolea ovata* – hairy hydrolea | x | | | x |
| *Lantana horrida* – Texas lantana | | | x | x |
| *Lithospermum caroliniense* – Puccon | | | x | |

Table 3 (Continued)

| Herbs (Continued) | Floodplain Hardwood Forest | Cypress Swamp | Upland Oak-Pine Forest | Streamside Woodlands |
|---|---|---|---|---|
| *Lobelia cardinalis* – Cardinal flower | x | | | x |
| *L. puberula* – downy lobelia | | | x | |
| *Mitchella repens* – Partridgeberry | | | x | x |
| *Mollugo verticillata* – green carpetweed | | | x | x |
| *Onoclea sensibilis* – sensitive fern | x | | | |
| *Opuntia spp.* – prickly pear | | | x | |
| *Osmunda regalis* – royal fern | x | | | |
| *Oxalis dillenii* – yellow wood sorrel | | | x | x |
| *O. violacea* – violet wood sorrel | | | x | |
| *Persicaria hydropiperoides* – swamp smartweed | | x | | x |
| *P. punctata* – water smartweed | | x | | |
| *Podophyllum peltatum* – common mayapple | | | x | |
| *Polianthes virginica* – false aloe | | | x | x |
| *Polygala polygama* – bitter milkwort | | | | x |
| *Polypodium polypodioides* – Resurrection fern | | | x | |
| *Rhexia mariana* – Maryland meadowbeauty | x | | | |
| *Salvia lyrata* – lyreleaf sage | | | x | x |
| *Sanicula canadensis* – Canada snakeroot | | | x | |
| *Scutellaria ovata* – egg skullcap | | | x | |
| *Senecio obovatus* – ragwort | | | x | |
| *Spermacoce glabra* – smooth buttonweed | | | x | |
| *Sprianthes gracilis* – Texas ladies' tresses | x | x | | x |
| *Stachys crenata* – shade betony | | | x | |
| *S. drummondii* – Drummond betony | | | x | |
| *Urtica urens* – dog nettle | x | | | x |
| *Verbesina virginica* – frostweed | | | x | x |
| *Vernonia texana* – Texas ironwood | | | x | |
| *Vicia levenworthii* – leavenworth vetch | | | x | |
| *Viola missouriensis* – Missouri violet | | | x | |
| *V. primulifolia* – violet | | | x | |
| *V. sagittata* – arrow-leaved violet | | | x | |
| *Xanthium strumarium* – cocklebur | x | | | x |
| **Grasses and Sedges** | | | | |
| *Andropogon glomeratus* – bushy bluestem | x | | x | x |
| *A. virginicus* – broomsedge | | | x | x |
| *Aristida purpurascens* – arrowfeather three-awn | | | x | x |
| *Arundinaria gigantea* – giant cane | x | | | x |
| *Axonopus affinis* – carpet grass | | | x | x |
| *Bromus unioloides* – rescue grass | | | x | x |
| *Carex amphibola* – amphibious sedge | x | | | |
| *C. cephalophora* – woodbank sedge | x | | | |
| *C. cherokeensis* – Cherokee sedge | x | | x | |
| *C. debilis* – spindlefruit sedge | | | x | |
| *C. hyalinolepis* – thinscale sedge | | | x | |
| *C. muhlenbergii* – Muhlenberg sedge | | | x | |
| *Chasmanthium latifolium* – inland sea oats | x | | | |
| *C. laxum* – wood grass | | | x | x |
| *C. sessiliflorum* – wood grass | | | x | x |
| *Digitaria filiformis* – slender crabgrass | | | | x |
| *Eleocharis obtusa* – blunt spikesedge | | | x | |

Table 3 (Continued)

| Grasses and Sedges (Continued) | Floodplain Hardwood Forest | Cypress Swamp | Upland Oak-Pine Forest | Streamside Woodlands |
|---|---|---|---|---|
| *Elymus canadensis* – Canada wildrye | | | x | x |
| *E. virginicus* – Virginia wildrye | | | x | x |
| *Erianthus contortus* – Bentawn plumegrass | x | | | |
| *Fimbristylis puberula* – Fimbry | | x | | |
| *Leersia virginica* – white grass | x | x | | |
| *Melica mutica* – two-flower melic | | | x | |
| *Muhlenbergia schreberi* – niblewill muhly | | | x | x |
| *Oplismenus hirtellus* – basketgrass | | | x | x |
| *O. setarius* – basketgrass | | | x | x |
| *Panicum anceps* – beaked panicum | x | | | |
| *P. gymnocarpon* – Savannah panicum | x | | | |
| *P. lanuginosum* – wooly panicum | | | x | x |
| *Paspalum bifidum* – pitchfork paspalum | | | x | |
| *P. dilatatum* – Dallis grass | | | x | x |
| *P. langei* – rustyseed paspalum | | | x | |
| *P. setaceum* – thin paspalum | | | x | |
| *Poa autumnalis* – autumn bluegrass | | | | x |
| *Schizachyrium scoparium* – little bluestem | | | | x |
| *Sorghastrum elliottii* – slender indiangrass | | | x | x |
| *Stipa avenacea* – blackseed needlegrass | | | x | |
| *Tridens flavus* – purpletop | | | x | |
| *Tripsacum dactyloides* – Eastern gamagrass | x | | | x |

TABLE 4

Checklist of Prairie Species in Chambless County

| Grasses and Sedges | Bluestem Prairie | Cordgrass Prairie |
|---|---|---|
| *Agrostis hyemalis* – spring bentgrass | x | |
| *A. scabra* – rough bentgrass | x | x |
| *Alopecurus carolinianus* – Carolina foxtail | x | |
| *Andropogon gerardi* – big blue stem | x | |
| *A. glomeratus* – bushy beard bluestem | x | x |
| *A. ternarius* – splitbeard bluestem | x | x |
| *A. virginicus* – broomsedge bluestem | x | |
| *Anthaenantia rufa* – purple silky scale | x | |
| *Aristida longespica* – slim spike three-awn | x | x |
| *A. oligantha* – prairie three-awn | x | |
| *A. purpurascens* – arrowfeather three-awn | x | |
| *Axonopus affinis* – carpet grass | x | x |
| *Bothriochloa ischaemum* – king ranch bluestem | x | |
| *B. saccaroides* – silver bluestem | x | x |
| *Briza minor* – little quaking grass | x | x |
| *Bromus unioloides* – rescue grass | x | x |
| *Carex spp.* – sedges | x | |
| *Cenchrus echinatus* – southern sandbur | x | |
| *C. incertus* – coast sandbur | x | |
| *Chloris petraea* – still-leaf chloris | x | x |
| *C. verticillata* – tumble windmill grass | x | x |
| *Cynodon dactylon* – Bermuda grass | x | x |

Table 4 (Continued)

| Grasses and Sedges (Continued) | Bluestem Prairie | Cordgrass Prairie |
|---|---|---|
| *Cyperus articulatus* – jointed flatsedge | x | x |
| *C. erythrorhizos* – red root flatsedge | x | |
| *C. esculentus* – chufa | x | |
| *C. globulosus* – Baldwin flatsedge | x | |
| *C. iria* – rice field sedge | x | |
| *C. strigosus* – false nut grass | x | x |
| *Dichanthium spp.* – gordo bluestem | x | |
| *Dichromena colorata* – white-top star rush | x | x |
| *Digitaria adscendens* – southern crabgrass | x | |
| *D. sanguinalis* – northern crabgrass | x | x |
| *Echinochloa crusgalli* – barnyard grass | x | x |
| *Eleocharis acicularis* – needle spikesedge | x | |
| *Eleusine indica* – goosegrass | x | |
| *Eragrostis lugens* – mourning lovegrass | x | x |
| *E. oxylepis* – red lovegrass | x | x |
| *Erianthus giganteus* – sugarcane plumegrass | x | |
| *Eriochloa contracta* – prairie supgrass | x | |
| *Fimbristylis spp.* – fimbry | x | |
| *Hordeum pusillum* – little barley | x | x |
| *Juncus effusus* – common rush | x | |
| *J. polycephalus* – flat leaf rush | x | |
| *Leptoloma cognatum* – fall witchgrass | x | x |
| *Muhlenbergia capillaris* – gulf muhly | x | |
| *Panicum anceps* – beaked panicum | x | |
| *P. capillare* – common witchgrass | x | |
| *P. filipes* – filey panicium | x | |
| *P. hians* – gaping panicium | x | |
| *P. lanuginosum* – woolly panicium | x | |
| *P. oligosanthes* – heller panicium | x | |
| *Paspalum dilatatum* – dallis grass | x | |
| *P. floridanum* – Florida paspalum | x | |
| *P. praecox* – cypress paspalum | x | |
| *P. notatum* – Bahia grass | x | x |
| *P. plicatulum* – brownseed paspalum | x | x |
| *P. setaceum* – thin paspalum | x | |
| *Phalaris caroliniana* – Carolina canarygrass | x | x |
| *Poa annua* – annual bluegrass | x | x |
| *Rhynchospora fascicularis* – stout beakrush | x | |
| *Schedonnardus paniculatus* – tumblegrass | x | x |
| *Schizachyrium scoparium* – little bluestem | x | x |
| *S. tenerum* – slender bluestem | x | |
| *Scleria spp.* – nutrush | x | |
| *Setaria geniculata* – knotroot bristlegrass | x | x |
| *S. glauca* – yellow foxtail | x | |
| *Sorghastrum avenaceum* – Indian grass | x | x |
| *Sorgum halepense* – Johnson grass | x | |
| *Spartina patens* – marshhay cordgrass | x | x |
| *S. pectinata* – prairie cordgrass | x | |
| *S. spartinae* – gulf cordgrass | x | x |
| *Sphenopholis obtusata* – prairie wedgescale | x | |
| *Sporobolus asper* – tall dropseed | x | |
| *S. indicus* – rattail smutgrass | x | x |
| *S. pyramidatus* – whorled dropseed | x | |
| *S. virginicus* – coastal dropseed | | x |

Table 4 (Continued)

| Grasses and Sedges (Continued) | Bluestem Prairie | Cordgrass Prairie |
|---|---|---|
| *Stenotaphrum secundatum* – St. Augustine grass | x | x |
| *Stipa leucotricha* – Texas winter-grass | x | x |
| *Tridens albescens* – white tridens | x | |
| *T. strictus* – longspike tridens | x | x |
| *Tripsacum dactyloides* – eastern gamagrass | x | |
| *Trisetum interruptum* – prairie trisetum | x | x |
| *Vulpia octoflora* – six-weeks fescue | x | x |
| *Willkommia texana* – Texas willkommia | x | |
| **Forbs** | | |
| *Agalinis maritima* – seaside gerardia | x | x |
| *A. purpurea* – | x | |
| *Allium spp.* – onion | x | x |
| *Ambrosia artemisiifolia* – eastern ragweed | x | |
| *A. psilostachya* – western ragweed | x | x |
| *A. trifida* – giant ragweed | x | |
| *Amsonia illustris* – blue star | x | |
| *Asclepias linearis* – slip milkweed | x | |
| *A. viridiflora* – green antelope-horn | x | x |
| *A. viridis* – antelope-horn | x | |
| *Aster ericoides* – Heath aster | x | |
| *A. subulatus* – salt marsh aster | x | x |
| *Baptisia leucantha* – plains wild indigo | x | |
| *B. sphaerocarpa* – green wild indigo | x | x |
| *Cacalia lanceolata* – Lance-leaf Indian plantain | x | |
| *Callirhoe involucrata* – poppy-mallow | x | |
| *Capsella bursa-pastoris* – shepperd's purse | x | |
| *Cassia fasciculata* – partridge pea | x | x |
| *C. obtusifolia* – sickle-pod senna | x | |
| *Castilleja indivisa* – Indian paintbrush | x | |
| *Centaurea americana* – basket-flower | x | |
| *Chenopodium album* – Lamb's-quarters | x | x |
| *Cirsium horridulum* – yellow thistle | x | |
| *C. texanum* – southern thistle | x | |
| *Clematis drummondii* – Texas virgin's bower | x | |
| *Commelina erecta* – erect dayflower | x | |
| *Convolvulus arvensis* – field bindweed | x | |
| *Conyza canadensis* – mare's tail | x | x |
| *Coreopsis cardaminaefolia* – Cardamine coreopsis | x | |
| *Croton capitatus* – woolly croton | x | x |
| *C. glandulosus* – northern croton | x | |
| *C. monanthogynus* – one-seeded croton | x | |
| *C. punctatus* – gulf croton | x | x |
| *Cuscuta cuspidata* – cusp dodder | x | x |
| *Desmanthus acuminatus* – sharp-pod bundleflower | x | |
| *D. illinoense* – Illinois bundleflower | x | x |
| *Dichondra carolinensis* – pony-foot | x | |
| *Erigeron geiseri* – basin fleabane | x | |
| *Erodium texanum* – Texas filaree | x | x |
| *Erythrina herbacea* – coral bean | x | x |
| *Eryngium hookeri* – hooker eryngo | x | x |
| *E. yuccifolium* – button snakeroot | x | |

Table 4 (Continued)

| Forbs (Continued) | Bluestem Prairie | Cordgrass Prairie |
|---|---|---|
| *Eupatorium coelestinum* – mist flower | x | |
| *E. compositifolium* – yankee weed | x | |
| *E. serotinum* – late flowerine eupatorium | x | x |
| *Euphorbia bicolor* – snow-on-the-prairie | x | x |
| *E. maculata* – spotted euphorbia | x | |
| *E. serpens* – mat euphorbia | x | |
| *E. spathulata* – warty euphorbia | x | |
| *Eustoma exaltatum* – tall prairie gentian | x | |
| *E. grandiflorum* – bluebell | x | x |
| *Eustylis purpurea* – purple pleatleaf | x | |
| *Evolvulus sericeus* – silky evolvulus | x | |
| *Gaillardia pulchella* – firewheel | x | x |
| *Gaura brachycarpa* – plain gaura | x | x |
| *G. parviflora* – small-flower gaura | x | |
| *Geranium carolinianum* – Carolina geranium | x | |
| *Gnaphalium obtusifolium* – fragrant cudweed | x | x |
| *Hedeoma hispidum* – rough hedeoma | x | |
| *Helenium amarum* – sneezeweed | x | x |
| *Helianthus angustifolius* – swamp sunflower | x | |
| *H. annuus* – common sunflower | x | x |
| *H. argophyllus* – silverleaf sunflower | x | |
| *H. hirsutus* – stiff-haired sunflower | x | |
| *Hydrocotyle verticillata* – whorled penny wort | x | |
| *Hydrolea ovata* – hairy hydrolea | x | |
| *Indigofera miniata* – coast indigo | x | |
| *I. suffruticosa* – indigo | x | |
| *Iva angustifolia* – narrowleaf sumpweed | x | x |
| *I. annua* – seacoast sumpweed | x | x |
| *Krameria lanceolata* – trailing rattany | x | x |
| *Krigia oppositifolia* – weedy dwarf dandelion | x | |
| *Lactuca canadensis* – wild lettuce | x | |
| *L. serriola* – prickly lettuce | x | |
| *Lantana horrida* – Texas lantana | x | |
| *Lepidium virginicum* – Virginia pepperweed | x | |
| *Lespedeza capitata* – round-head bush clover | x | |
| *L. virginica* – slender bush clover | x | |
| *Liatris acidota* – sharp gayfeather | x | |
| *L. elegans* – pinkscale gayfeather | x | |
| *L. pycnostachya* – Kansas gayfeather | x | |
| *Linum rigidum* – stiffstem flax | x | x |
| *Lobelia appendiculata* – earflower lobelia | x | |
| *Machaeranthera phyllocephala* – camphor daisy | x | x |
| *Medicago arabica* – spotted bur-clover | x | |
| *M. lupulina* – black medick | x | |
| *M. polymorpha* – California bur-clover | x | x |
| *Melilotus albus* – white sweet clover | x | |
| *M. officinalis* – yellow sweet clover | x | |
| *Mimosa strigillosa* – powderpuff | x | |
| *Mollugo verticillata* – green carpetweed | x | |
| *Monarda fistulosa* – wild bergamont | x | |
| *Neptunia lutea* – low neptunia yellow-puff | x | x |
| *N. pubescens* – tropical nuptunia | x | |
| *Nothoscordum bivalve* – crow-poison | x | x |

Table 4 (Continued)

| Forbs (Continued) | Bluestem Prairie | Cordgrass Prairie |
|---|---|---|
| *Oenothera laciniata* – cut-leaved evening primrose | x | |
| *O. speciosa* – pink evening primrose | x | |
| *Oxalis dillenii* – yellow wood-sorrel | x | |
| *O. violacea* – violet wood-sorrel | x | |
| *Palafoxia rosea* – rose palafoxia | x | |
| *Parthenium hysterophorus* – false ragweed | x | x |
| *Passiflora incarnata* – Maypop | x | |
| *Persicaria hydropiperoides* – swamp smartweed | x | |
| *P. punctatum* – dotted smartweed | x | |
| *Phyla nodiflora* – common frog-fruit | x | |
| *Physalis angulata* – ground cherry | x | |
| *Physostegia intermedia* – intermediate lion's heart | x | |
| *Phytolacca americana* – pokeweed | x | |
| *Plantago elongata* – slender plantain | x | |
| *P. rhodosperma* – red-seeded plantain | x | |
| *Pluchea foetida* – stinking fleabane | x | |
| *P. rosea* – marsh fleabane | x | |
| *Polanisia dodecandra* – clammy-weed | x | |
| *Polygonum aviculare* – knotweed | x | x |
| *Portulaca mundula* – shaggy portulaca | x | |
| *Pyrrhopappus multicaulis* – many stem false dandelion | x | |
| *Ranunculus muricatus* – rough seed buttercup | x | |
| *Ratibida peduncularis* – Mexican hat | x | |
| *Rhexia mariana* – Maryland meadowbeauty | x | |
| *Rhynchosia texana* – Texas snoutbean | x | |
| *Rudbeckia grandiflora* – large flower coneflower | x | |
| *R. hirta* – black-eyed Susan | x | |
| *Ruellia humilis* – low ruellia | x | |
| *Rumex crispus* – curly dock | x | |
| *R. pulcher* – fiddle dock | x | x |
| *Sabatia campestris* – prairie rose-gentian | x | x |
| *Salvia coccinea* – tropical sage | x | |
| *S. lyrata* – lyreleaf sage | x | |
| *Samolus ebracteatus* – coast brookweed | x | x |
| *Schrankia uncinata* – catclaw sensitive brier | x | |
| *Scutellaria drummondii* – Drummond skullcap | x | |
| *Senecio imparipinnatus* – groundsel | x | |
| *Sesbania marcrocarpa* – bequilla | x | |
| *S. vesicaria* – bag-pod sesbania | | |
| *Sida lindheimeri* – showy sida | x | |
| *S. rhombifolia* – arrowleaf sida | x | |
| *S. spinosa* – prickly mallow | x | |
| *Silene antirrhina* – sleepy catchfly | x | |
| *Silphium asperrimum* – rough stem rosin-weed | x | |
| *Sisyrinchium angustifolium* – blue-eyed grass | x | |
| *S. exile* – blue-eyed grass | x | |
| *Solanum elaeagnifolium* – silver-leaf nightshade | x | x |
| *S. rostratum* – buffalo bur | x | |
| *Sonchus asper* – common sow thistle | x | |
| *S. oleraceus* – sow thistle | x | |
| *Spigelia texana* – Texas pinkroot | x | |
| *Spiranthes cernua* – nodding ladies' tresses | x | |
| *Stellaria media* – chick weed | x | |

Table 4 (Continued)

| Forbs (Continued) | Bluestem Prairie | Cordgrass Prairie |
|---|---|---|
| *Stillingia sylvatica* – queen's delight | x | |
| *Strophostyles helvola* – trailing wildbean | x | |
| *Tephrosia lindheimeri* – tephrosia | x | |
| *T. onobrychoides* – multi-bloom tephrosia | x | |
| *Thelesperma spp.* – greenthread | x | |
| *Tidestromia lanuginosa* – woolly tidestromia | x | |
| *Tillaea aquatica* – water pygmy-weed | x | |
| *Tradescantia ohioensis* – Ohio spiderwort | x | |
| *Tragia glanduligera* – roseburn | x | |
| *Trifolium amphianthum* – peanut clover | x | |
| *T. repens* – white clover | x | |
| *Triodanis perfoliata* – Venus' looking-glass | x | |
| *Verbena halei* – Texas vervain | x | |
| *V. hastata* – blue vervain | x | |
| *V. xutha* – Gulf vervain | x | |
| *Vicia leavenworthii* – leavenworth vetch | x | |
| *V. ludoviciana* – Louisiana vetch | x | |
| *Vigna luteola* – cowpea | x | x |
| *Xanthocephalum texanum* – Texas broomweed | x | x |
| **Woody Plants** | | |
| *Acacia farnesiana* – sache | x | x |
| *Amorpha fruticosa* – bastard indigo | x | |
| *Asimina triloba* – paẇpaw | x | |
| *Ascyrum hypericoides* – St. Andrew's cross | x | |
| *Baccharis halimifolia* – sea myrtle | x | x |
| *Bumelia lanuginosa* – gum bumelia | x | x |
| *Cornus drummondii* – roughleaf dogwood | x | |
| *Diospyros virginiana* – common persimmon | x | |
| *Ilex vomitoria* – yaupon | x | |
| *Iva frutescens* – bigleaf sumpweed | x | x |
| *Melia azedarach* – Chinaberry tree | x | |
| *Myrica cerifera* – southern wax-myrtle | x | |
| *Opuntia lindheimeri* – Texas prickly pear | x | x |
| *O. macrorhiza* – plains prickly pear | x | |
| *Parkinsonia aculeata* – retama | x | |
| *Quercus marilandica* – blackjack oak | x | |
| *Rosa bracteata* – MacCartney rose | x | |
| *Rubus louisianus* – blackberry | x | |
| *R. trivialis* – Southern dewberry | x | |
| *Sapindus saponaria* – Western soapberry | | |
| *Sapium sebiferum* – Chinese tallow | x | |
| *Sesbania drummondii* – rattlebox | x | |
| *Tamarix gallica* – salt cedar | x | |
| *Zanthoxylum clava – herculis* – toothache tree | x | |
| *Z. fagara* – lime prickly ash | x | |

TABLE 5

Checklist of Marsh Species in Chambers County

| Grasses – Sedges – Rushes | Fresh Marsh | Brackish Marsh |
|---|---|---|
| *Brachiaria platyphylla* – broadleaf signalgrass | x | |
| *Carex amphibola* – Amphibious sedge | x | |
| *C. vulpinoidea* – fox sedge | x | |

Table 5 (Continued)

| Grasses – Sedges – Rushes (Continued) | Fresh Marsh | Brackish Marsh |
|---|---|---|
| *Cladium jamaicense* – Jamica sawgrass | x | |
| *Cyperus acuminatus* – tapeleaf sedge | x | |
| *C. articulatus* – jointed sedge | x | x |
| *C. ochraceus* – flatsedge | x | |
| *C. virens* – green flatsedge | x | |
| *Distichlis spicata* – seashore saltgrass | | x |
| *Echinochloa colonum* – jungle rice | x | |
| *E. crusgalli* – barnyard grass | x | x |
| *E. cruspavonis* – Gulf cockspur | x | x |
| *Eichhornia crassipes* – water hyacinth | x | x |
| *Eleocharis acicularis* – needle spikesedge | x | |
| *E. cellulosa* – Gulfcoast spikesedge | x | x |
| *E. montevidensis* – sand spikesedge | x | |
| *E. parvula* – dwarf spikesedge | | x |
| *E. quadrangulata* – squarestem spikesedge | x | x |
| *Eragrostis reptans* – lovegrass | x | |
| *Fimbristylis castanea* – fimbry | x | x |
| *F. vahlii* – Vahl fimbry | x | x |
| *Juncus acuminatus* – knotleaf rush | x | |
| *J. bufonius* – toad-rush | x | |
| *J. effusus* – common rush | x | |
| *J. interior* – interior rush | x | |
| *J. roemerianus* – needlegrass rush | | x |
| *J. validus* – roundhead rush | x | |
| *Leersia lenticularis* – catchfly grass | x | |
| *L. monandra* – bunch cutgrass | x | |
| *L. virginica* – white grass | x | |
| *Leptochloa filiformis* – red sprangletop | x | x |
| *L. nealleyi* – Nealley sprangletop | x | x |
| *L. uninervia* – Mexican sprangletop | x | x |
| *Monanthochloë littoralis* – shoregrass | | x |
| *Panicum anceps* – beaked panicum | x | |
| *P. geminatum* – | x | |
| *P. hemitomon* – maidencane | x | |
| *P. paludivagum* – water panicum | x | x |
| *P. repens* – torpedo grass | x | x |
| *P. virgatum* – switchgrass | x | x |
| *Paspalum acuminatum* – brook paspalum | x | |
| *P. bifidum* – pitchfork paspalum | x | |
| *P. distichum* – knotgrass | x | x |
| *P. lividum* – longtom | x | x |
| *P. monostachyum* – Gulfdune paspalum | | x |
| *P. vaginatum* – seashore paspalum | | x |
| *Phalaris caroliniana* – Carolina canarygrass | x | x |
| *Phragmites communis* – common reed | x | x |
| *Polypogon monspeliensis* – rabbitfoot grass | x | x |
| *Rhynchospora corniculata* – horned beakrush | x | x |
| *Sacciolepis striata* – American cupscale | x | |
| *Scirpus americanus* – American bulrush | | x |
| *S. californicus* – California bulrush | x | x |
| *S. maritimus* – salt-marsh bulrush | | x |
| *S. olneyi* – Olney bulrush | | x |
| *Scleria sp.* – nutrush | x | |

Table 5 (Continued)

| Grasses – Sedges – Rushes (Continued) | Fresh Marsh | Brackish Marsh |
|---|---|---|
| *Setaria geniculata* – knotroot bristlegrass | x | x |
| *S. Magna* – giant bristlegrass | | |
| *Spartina alterniflora* – smooth cordgrass | | x |
| *S. cynosuroides* – big cordgrass | | x |
| *S. patens* – marshhay cordgrass | x | x |
| *S. spartinae* – Gulf cordgrass | x | x |
| *Sporobolus virginicus* – seashore dropseed | | x |
| *Zizaniopsis miliacea* – giant cutgrass | x | |
| **Forbs** | | |
| *Acnida tamariscina* – Nuttall's water-hemp | | |
| *Alternanthera philoxeroides* – Alligator-weed | x | |
| *Amaranthus greggii* – Gregg amaranthus | x | |
| *A. tamariscinus* – water hempweed | x | x |
| *Ammannia coccinea* – purple ammania | x | |
| *Asclepias perennis* – shore milkweed | x | |
| *Aster spinosus* – devilweed aster | x | x |
| *A. subulatus* – slim aster | x | x |
| *A. tenuifolius* – saline aster | | x |
| *Azolla caroliniana* – water fern | x | |
| *Bacopa monnieri* – water-hyssop | x | x |
| *B. rotundifolia* – disc water-hyssop | x | |
| *Borrichia frutescens* – bushy sea oxeye | | x |
| *Cakile fusiformis* – sea rocket | | x |
| *Callitriche heterophylla* – large waterwort | x | |
| *C. terrestris* – annual waterstarwort | x | |
| *Calystegia sepium* – hedge-bindweed | x | |
| *Canna glauca* – Indian-shot | x | |
| *Cardiospermum halicacabum* – common balloon-vine | x | x |
| *Centaurium calycosum* – Buckley centaury | x | |
| *Centunculus minumus* – chaffweed | x | |
| *Corchorus hirtus* – Orinoco jute | x | |
| *Desmanthus illinoensis* – Illinois bundleflower | x | x |
| *Diodia virginiana* – rough buttonweed | x | |
| *Echinodorus cordifolius* – burhead | x | x |
| *E. parvulus* – burhead | x | x |
| *E. rostratus* – burhead | x | x |
| *Eclipta alba* – Yerba de tajo | x | |
| *Glinus radiatus* – | x | |
| *Heliotropium curassavicum* – seaside heliotrope | | x |
| *H. procumbens* – spike heliotrope | | x |
| *Heteranthera limosa* – blue mud-plantain | x | |
| *H. reniformis* – mud-plantain | x | |
| *Hydrocotyle bonariensis* – largeleaf pennywort | x | x |
| *H. umbellata* – Umbrella pennywort | x | |
| *Hydrolea ovata* – hairy hydrolea | x | |
| *Hymenocallis liriosme* – fragrant white spiderlily | | x |
| *Ipomoea sagittata* – salt marsh morning glory | | x |
| *I. trichocarpa* – sharppod morning glory | x | |
| *Iris virginica* – Southern blueflag | x | |
| *Justicia lanceolata* – lance-leaved water willow | | |
| *Kosteletzkya virginica* – saltmarsh mallow | | x |

Table 5 (Continued)

| Forbs (Continued) | Fresh Marsh | Brackish Marsh |
|---|---|---|
| *Lemna gibba* – swollen duckweed | x | |
| *Limnobium spongia* – common frog's-bit | x | |
| *Limonium nashii* – Carolina sea-lavender | | x |
| *Lindernia anagallidea* – false pimpernel | x | |
| *Lobelia cardinalis* – Cardinal flower | x | |
| *Ludwigia glandulosa* – creeping seedbox | x | |
| *L. octovalvis* – water-primrose | x | |
| *L. palustris* – marsh purslane | x | x |
| *L. peploides* – verdolaga de agua | x | |
| *Machaeranthera phyllocephala* – camphor daisy | x | x |
| *Malachra capitata* – Malva de caballo | x | |
| *Malvastrum coromandelianum* – threelobe falsemallow | x | |
| *Melochia corchorifolia* – redweed | x | |
| *Modiola caroliniana* – Carolina modiola | x | |
| *Myriophyllum pinnatum* – green parrot's-feather | x | |
| *Najas guadalupensis* – Southern naiad | x | |
| *N. mariana* – spiny naiad | x | |
| *Persicaria coccinea* – big root smartweed | x | |
| *P. hydropiperoides* – swamp smartweed | x | |
| *P. punctata* – dotted smartweed | x | |
| *Physostegia angustifolia* – lion's heart | x | |
| *P. intermedia* – intermediate lion's heart | x | |
| *Pistia stratiotes* – water-lettuce | x | |
| *Pluchea purpurascens* – purple pluchea | x | x |
| *P. rosea* – marsh fleabag | x | |
| *Polygonum aviculare* – Prostrate knotweed | x | |
| *Potamogeton diversifolius* – waterthread pondweed | x | |
| *P. nodosus* – longleaf pondweed | x | |
| *Proserpinaca palustris* – mermaid-weed | x | |
| *Rivina humilis* – rougeplant | x | |
| *Rorippa islandica* – bog marshcress | x | |
| *Rumex chrysocarpus* – dock | x | |
| *R. pulcher* – fiddle dock | x | x |
| *Ruppia maritama* – widgeon-grass | x | |
| *Sabatia campestris* – prairie rose-gentian | x | |
| *Sagittaria graminea* – grassy arrowhead | x | |
| *S. latifolia* – common arrowhead | x | |
| *S. longiloba* – longlobe arrowhead | x | x |
| *S. papillosa* – nipplebract arrowhead | x | |
| *Samolus ebracteatus* – coast brookweed | x | x |
| *S. parviflorus* – brookweed | x | x |
| *Sesuvium maritimum* – sea purslane | | x |
| *S. portulacastrum* – sea purslane | x | x |
| *Solanum nodiflorum* – nightshade | x | |
| *Solidago sempervirens var. Mexicana* – seaside goldenrod | x | x |
| *Spergularia echinosperma* – bristleseed sand-spurry | | x |
| *S. marina* – marsh sand-spurry | | x |
| *Sphenoclea zeylanica* – pie fruit | x | |
| *Suaeda linearis* – annual seepweed | | x |
| *Teucrium canadense* – woodsage | x | |
| *Thelesperma filifolium* – green-thread | x | |
| *Thurovia triflora* – three flowered thurovia | x | x |

Table 5 (Continued)

| Forbs (Continued) | Fresh Marsh | Brackish Marsh |
|---|---|---|
| *Typha angustifolia* – narrow-leaved cattail | x | x |
| *T. latifolia* – common cattail | x | |
| *Utricularia gibba* – cone-spur bladderwort | x | |
| *U. purpurea* – purple bladderwort | x | |
| *Wolffia columbiana* – Columbia water-meal | x | |
| *Wolffiella gladiata* – mud-midget | x | |
| *Xanthium strumarium* – cocklebur | x | |
| **Woody Plants** | | |
| *Atriplex spp.* – saltbush | | x |
| *Baccharis halimifolia* – sea myrtle | x | x |
| *Batis maritima* – saltwort | | x |
| *Bumelia lanuginosa* – gum bumelia | x | |
| *Cephalanthus occidentalis* – common buttonbush | x | |
| *Diospyros texana* – Mexican persimmon | x | |
| *Hibiscus lasiocarpos* – woolly rose-mallow | x | x |
| *H. militaris* – scarlet rose-mallow | x | |
| *Iva frutescens* – bigleaf sumpweed | | x |
| *Lycium carolinianum* – Carolina wolfberry | | x |
| *Salicornia bigelovii* – biglow glasswort | | x |
| *S. virginica* – Virginia glasswort | | x |
| *Sapium sebiferum* – Chinese tallow | x | |
| *Sesbania drummondii* – rattlebox | x | x |
| *S. macrocarpa* – coffeebean | x | x |
| *Tamarix gallica* – salt cedar | x | x |
| *Zanthoxylum clava-herculis* – toothache tree | x | x |

## PLANT ASSEMBLAGES OF CHAMBERS COUNTY

### *Oak-Pine Forest*

Oak-pine forests are identified by presence of loblolly pine in relative abundances of 25-100%. Associated species usually include sweetgum, (see Table 2 for scientific names), willow oak, and water oak. Less frequently, laurel oak, basket oak, southern red oak, or hickories may be encountered. Forest height ranges from 20 to 30 m; tree density is in the vicinity of 500 trees per ha (trees >10 cm diameter at breast ht.); basal area is 20 to 30 $m^2$/ha. The understory shrubs may be dense or sparse, depending on height and density of the tree canopy. Yaupon and American beautyberry are predominant shrubs. Greenbriar, trumpet creeper and virginia creeper are the common vines.

In Chambers County, oak-pine forests occur on upper terraces of the Trinity River floodplain, along the major streams emptying into upper Trinity Bay (Double Bayou, Turtle Bayou), and along Cedar Bayou down to Baytown. This assemblage is replaced by floodplain hardwood forest or cypress swamp on the lower terraces of the Trinity River. Under natural conditions, the boundary between oak-pine forest and grassland may have been determined by soil differences,

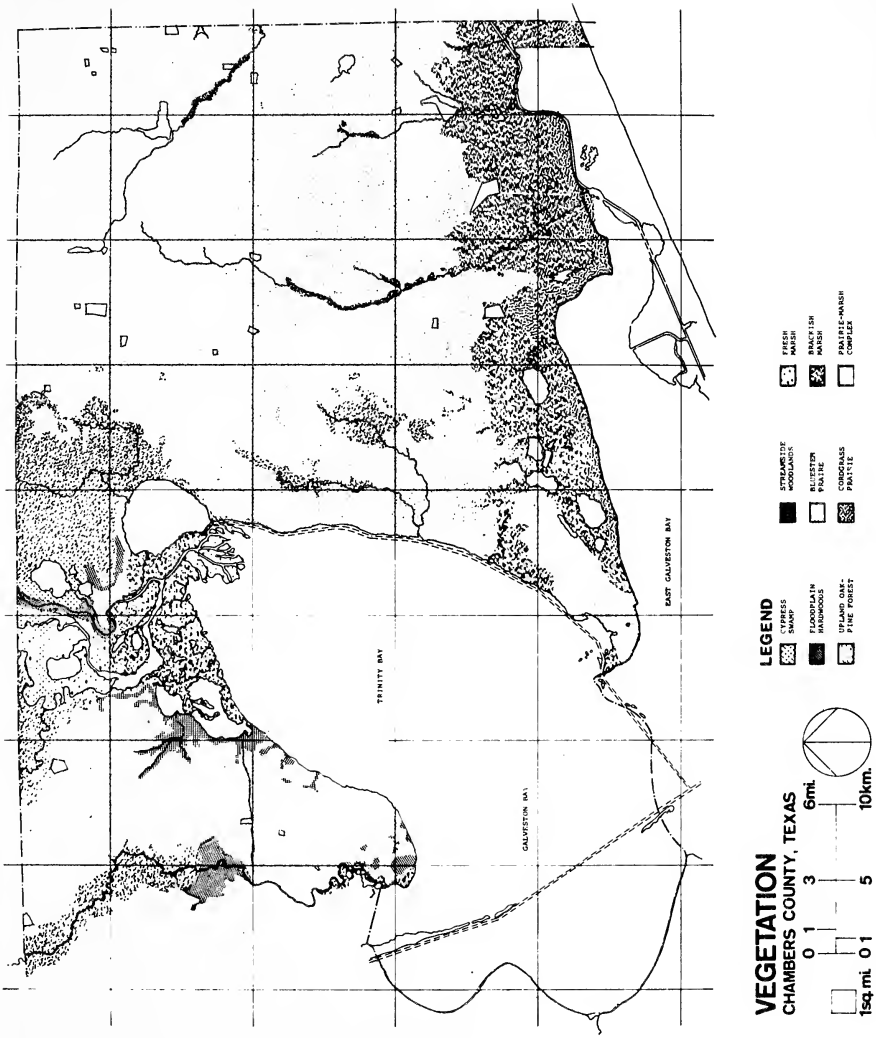


Figure 1. Map of Chambers County Vegetation.

since forests are often limited to alluvial soils with better drainage and a more constant moisture supply (Kilburn, 1959; Severson and Arneman, 1973). Periodic drought and subsequent fires may also have played a role in determining this vegetation boundary, but our observation of successional patterns in the prairie and our unpublished data from Harris County suggest that the soil difference is of primary importance in Chambers County.

Edmiston (1963), Monk (1965), and others indicate that forests such as these are often successional – the predominance of loblolly pine and sweetgum is indicative of fire or cutting – and that 75-150 yrs after disturbance they will revert

to mixed forests containing much less pine. On relatively dry sites in Chambers County, southern red oak, post oak and hickories will probably become more prominent; on moist sites magnolia and basket oak probably will predominate. This assemblage probably represents the southernmost extension of the Southern Mixed Hardwood Forest (Monk, 1965). McLeod (1971) calls it the "Lower Big Thicket Forest" and Küchler (1964) maps it as Oak-Hickory-Pine Forest.

The oak-pine forest along both forks of Double Bayou was erroneously mapped as oak motte by Fisher, *et al.,* (1972). Fisher, *et al.,* (1972) also mistakenly included shortleaf and longleaf pine as components of the mixed Pine-Hardwood Forest, which roughly corresponds to our oak-pine forest. Neither species of pine is present in Chambers County.

*Cypress Swamp*

Cypress swamps are relatively open stands of bald-cypress about 30 m high, with high tree density (about 500 per ha) and high basal area (50 $m^2$/ha). Other important woody species include willow, water elm, and buttonbush. Along the edges of the cypress swamps, water hickory is common.

Cypress swamps are common in the Trinity River floodplain where standing fresh water exists for most of the year. Cypress trees appear to be very sensitive to saline encroachment (O'Neil, 1949), so they do not extend onto the present Trinity delta. Since regeneration appears to occur only when the swamp is dry (Hall, *et al.,* 1946) permanent flooding could have a substantial impact on these forests.

Cypress swamps are well-known in the southeastern United States (Penfound, 1952), and those in Chambers County appear to be typical.

*Floodplain Hardwood Forest*

Water oak, cedar elm, sugarberry, green ash, and hawthorn characterize the forests of the lower Trinity River floodplain and the mouth of Cedar Bayou. Willow and sycamore are common on recent sand bars. Cypress and tupelo occur in the sloughs. The forests are relatively open, with widely spaced overstory oaks above a subcanopy of cedar elm, sugarberry, and hawthorn (15-20 m tall). Tree density is about 700 stems per ha; basal area is about 15 $m^2$ per ha. In northern Chambers County, the understory varies from dense stands of palmetto to continuous grass and herb cover. Savannah panicum, smartweed and water willow are dominant in the wet grassy areas; woodgrass and sedges dominate in drier grassy areas. Along the Trinity Bay margin and at the mouth of Cedar Bayou, palmetto is replaced by dense stands of yaupon, and the herb layer contains more upland grasses and herbs. Vines are a conspicuous component everywhere. Rattan, poison ivy and trumpet creeper are common in the Trinity floodplain; greenbriar and blackberry are important along the Trinity Bay margin and at the mouth of Cedar Bayou.

Increased seasonal flooding in floodplain forests usually enhances tree growth, but permanent flooding is detrimental to many floodplain hardwood species (Broadfoot and Williston, 1973). Saline encroachment may also influence these forests: we observed tree death at the mouth of Cedar Bayou, apparently due to subsidence-related salt encroachment.

Küchler (1964) maps the Trinity River floodplain as Southern Floodplain Forest. However, the species composition is quite different and does not fit any of Küchler's mapping units. The Trinity River floodplain forest bears a stronger resemblance to the vegetation along rivers further south along the central Texas coast described by Tharp (1926).

*Streamside Woodlands*

Sugarberry, hawthorn, and low-growing water oak are the characteristic species in the streamside woodlands. The trees are usually small (less than 15 m tall), and are frequently tied together in a dense tangle by greenbriar and blackberry.

Streamside woodlands occur along Oyster Bayou, Elm Bayou and Spindletop Bayou. In contrast, the larger bayous emptying into Trinity Bay support oak-pine forest along the streambanks. Streamside woodlands are a conspicuous feature of prairie regions (Weaver, 1960).

*Bluestem Prairie*

Characteristic species of this assemblage are big bluestem, Indiangrass, switchgrass, little bluestem, and eastern gamagrass. Under natural conditions, these species form a uniform stand of grasses 1-2 m tall, interrupted by individuals and clumps of legumes and other forbs. In Chambers County, species composition appears to vary with soil texture. Little bluestem predominates on sandy soils that are drier; the others are more common on moist sites (clay and loam soil). Brownseed paspalum is also common on moist sites. The Soil Conservation Service (1976) divides this assemblage into 4 types on the basis of soil texture (sandy prairie, loamy prairie, blackland prairie, lowland prairie). However, species composition differences are not great, so we prefer to lump them into a single assemblage.

Today, this assemblage has been almost completely replaced in eastern Chambers County by rice fields and pastures. The fields are normally planted 2 years in rice and then the fallow for 2-3 years. The first year following cultivation, sedges, slim aster, and barnyard grass are dominant. Subsequently, bushybeard, annual sumpweed, rattlebush, and sea myrtle invade. On heavily grazed sites, carpetgrass and smutgrass predominate. Vasey grass becomes dominant on properly grazed sites and may persist for many years. In western Chambers County most of the prairie is no longer in agricultural use, but due to protection from fire, extensive brushland has emerged. Sea myrtle is the dominant brush species. Chinese tallow and huisache are common in some areas.

This assemblage is the same as the Eastern Tallgrass Prairie Formation of Weaver (1954) or the Bluestem Prairie of Küchler (1964). Küchler (1964) mapped it

as Bluestem-Sacahuiste Prairie, but since sacahuiste (Gulf cordgrass) is restricted to the coastal rim in Chambers County, we prefer to designate this assemblage as Bluestem Prairie.

*Cordgrass Prairie*

Gulf Cordgrass (sacahuiste) is the characteristic species of the cordgrass prairie, though little bluestem may also occur. This assemblage was probably once more extensive as an intermediate type between upland prairie and brackish marsh, but it now exists primarily on the natural levee along the north edge of East Bay as a nearly pure stand of Gulf cordgrass. It is equivalent to the Salty Prairie Range Site of the Soil Conservation Service.

Cordgrass prairie is more extensive further south along the Texas Coast; other cordgrass species are common on saline soils of northern prairies (Weaver, 1954).

*Fresh Marsh*

Marshland can be divided into shallow and deep phases of fresh, brackish, and salt assemblages (Shaw and Fradine, 1956; Shiftlet, 1963). However, here the only divisions we recognize are fresh and brackish marsh, primarily because the others are either absent or are of very limited extent in Chambers County. The principal distinguishing feature is presence (in brackish marsh) or absence (in fresh marsh) of marsh-hay cordgrass (*Spartina patens*).

The most common species of fresh marshes are common reed, sawgrass, cutgrass, torpedograss, longtom, cattail, and big cordgrass, in various combinations. Alligator weed is a common successional species. It occurs on the active areas of the Trinity River delta between Interstate Highway 10 and the northern boundary of the county.

As mentioned earlier, landward sides of the coastal marshes, especially near the mouth of Oyster Bayou, once supported extensive fresh marsh assemblages (USDI, 1952). Large stands of common reed may, in fact, have persisted until the mid 1950's (F. M. Fisher, personal communication). However, at present there is little evidence of fresh marsh vegetation. At least 3, and possibly 4, factors are involved. First, freshwater marsh on Beaumont Clay soils has been claimed for rice production. Second, impoundment of water for rice farming probably has reduced freshwater input into the marshes. Third, land subsidence probably has resulted in increased salt water intrusion. Fourth, disturbances (e.g., burning to improve muskrat habitat) seems to favor fresh marsh species (O'Neil, 1949), so changed management objectives may have resulted in succession from fresh-marsh species to brackish-marsh species without any actual change in soil or water salinity. Marsh drainage and conversion to rice fields are undoubtedly the most significant factors.

*Brackish Marsh*

This assemblage is dominated by marsh-hay cordgrass and/or seashore saltgrass. Usually one or more rush or sedge species occur in isolated clumps (especially

salt-marsh bulrush and olney bulrush). Near tidal drains needlegrass rush may occur. On levees, fresh-marsh species, especially common reed and big cordgrass, are common. Seashore paspalum and longtom are also common in the fresher areas.

Smooth cordgrass is common in Chambers County, but it occupies such a narrow fringe along East Galveston Bay that it is not mappable as a distinct assemblage.

Brackish marsh is the predominant marsh assemblage in Chambers County. It occurs in the mouth of Cedar Bayou, in the Trinity River Bottom south of Interstate Highway 10, along Smith Point, and all along the southeastern part of the county.

Any alteration of overland water flow will undoubtedly affect species composition and productivity in the coastal marshes. The area of the marshes has probably now been reduced by drainage and reclamation as much as is possible, mostly at the expense of the fresh-marsh types, so further reduction of fresh water input will result in slightly increased salinity, and possibly increased frequency of dry conditions in the marsh. This probably will reduce productivity and may result in a shift in species composition toward gulf cordgrass.

Brackish marsh appears equivalent to Küchler's (1964) Northern Cordgrass Prairie (marsh-hay cordgrass and seashore saltgrass dominant). It bears little relationship to his Southern Cordgrass Prairie (smooth cordgrass dominant). Hence we believe the term brackish marsh is a more appropriate name until additional data are published on the relationship of Küchler's Northern Cordgrass Prairie type to the southern marshes. This marsh type is probably predominant from the Trinity River eastward at least to the Mississippi (Chabreck, 1972).

The extreme southeastern tip of the county fronts on Galveston Bay, and supports dune vegetation. Because of the small area involved, and the presence of brackish marsh species in the assemblage, the area is included as brackish marsh. It is actually a distinct assemblage, dominated by bitter panicum and marsh-hay cordgrass. It has much in common with the dune vegetation of Mustang Island, Texas (Gillespie, 1976).

## VEGETATION – ENVIRONMENT RELATIONSHIPS

Water is the factor with most obvious influence on the vegetation of Chambers County. The boundaries between marsh and prairie are set by limits of periodic flooding, and the boundary between prairie and forest is indirectly related to water through the effect of soil moisture in preventing upland prairie fires from burning into lowland forest. Water is important in determining variation within the physiognomic types, also, though other factors may also be involved. In marshes, variation in water level and salinity can account for most of the variation in species composition (Penfound and Hathaway, 1938; Shiflet, 1963).

In forests and prairies, water availability depends on topographic position and on soil texture, so these landscape factors are the primary determinants of species

composition (Caplenor, 1968; Rice, 1965; Weaver, 1954; Wells, 1942). The moisture dependence is readily apparent in the forests of Chambers County, where 4 of the 5 forest assemblages (cypress swamp, floodplain hardwood, flatwoods, and streamside woodlands) can be identified from topographic information alone.

In the prairies, variation in species composition is less apparent—certainly vegetation physiognomy changes much less—with changes in moisture availability.

Species composition may also be influenced by catastrophic events like fires or floods. For example, long-term fluctuations in climate may cause marked changes in prairie species composition (Albertson and Tomanek, 1965; Lynch, 1971), or storm tide flooding which brings in salt water can affect species composition of marshes (Shiflet, 1963). Thus, species composition of plant assemblages is probably constantly changing in response to long- and short-term climatic fluctuations.

In addition to responding to environmental factors, vegetation also responds to human disturbance. All stages of prairie, forest, and marsh succession can be found in Chambers County, and they are mentioned briefly in the climax assemblage descriptions above.

None of the variation in physiognomy or species composition of Chambers County vegetation is obviously tied to soil chemical differences (apart from salinity), though variations in soil nutrients (i.e., variations in fertility) undoubtedly exist and influence growth rates and species composition within assemblages.

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# GEOMYID INTERACTION IN BURROW SYSTEMS

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ABSTRACT

A 5.5 m long and 7.5 mm wide glass-walled observation chamber restricted construction of burrow systems to 2 dimensions. Introductions of representatives of 3 genera of North American geomyids were made in various combinations: size was an important factor in combat. Geomyids were dominant over other vertebrates and invertebrates tested. Ecological factors are probably more important than aggressiveness in determining range boundaries for pocket gophers.

## INTRODUCTION

A variety of parasitic and nonparasitic invertebrates, herptiles, and small mammals invade burrow systems. The responses between pocket gophers and this diverse fauna is speculative. Documentation of several adult pocket gophers in a single burrow system is sparse. Nonetheless, burrow systems do intersect and cases of plural occupancy reported (Hansen and Miller, 1959). Encounters within burrow systems probably occur regularly in areas of high population density. The degree and nature of intra- and interspecific interaction is little known, even though the aggressive nature of geomyids is demonstrated in that pocket gophers are solitary. Interspecific aggressiveness between pocket gopher species may be important in mutual competitive exclusion and parapatry characteristic of geomyids.

Lack of knowledge in these areas revolves around the difficulty of visually monitoring the subterranean activities of this most fossorial of rodent families in North America. This study attempted to solve this problem by construction of a large dirt-filled, glass-walled observation chamber which permitted construction of a burrow system in only 2 dimensions.

## MATERIALS AND METHODS

The burrow simulator measured 5.5 m long, 1.2 m high, and 7.6 cms wide (Fig 1). Construction of a similar observation chamber has been described by

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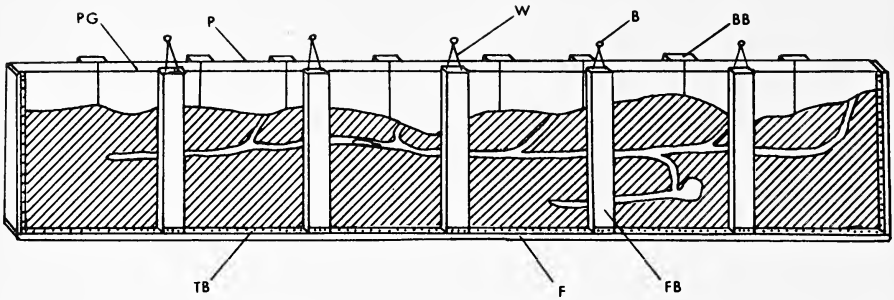


Figure 1. Position of components used in the construction of the burrow simulator. B = bolt, BB = back brace, F = frame, FB = front brace, P = plywood, PG = plate glass, TB = triangular brace, W = wire. Soil within the chamber was never changed, remaining "fresh" after a year and a half of use.

Hendricksen and McGaugh (1975) after a design by Hickman (1974). Pocket gopher movements and interactions were observed in the chamber with 2 inflourescent and 2 incandescent infra-red lamps.

Pocket gophers were live-trapped (Baker and Williams, 1972): *Thomomys bottae* from localities near Sacramento, New Mexico; *Geomys bursarius* from Kermit, and Slaton, Texas; and *Pappogeomys castanops* from Lubbock, Texas. Animals were fed crimped oats and laboratory rabbit chow, occasionally lettuce, and individually caged in covered 20 gal garbage cans filled with moist sandy soil.

A minimum of 3 observations of individuals of each species weight class was made for inter- and intraspecific interaction of pocket gophers, and for each geomyid interaction with various non-geomyid vertebrates and invertebrates. Pocket gophers were permitted 3 to 5 days in the burrow simulator before being considered resident.

## RESULTS

Pocket gophers yielded no sign of ticks, fleas, warbles, or any evidence of infection. Mites were found on pocket gophers, but the animals did not appear irritated by their presence.

Various beetles, size 20 to 40 mm, were ignored in the burrow system. Camel crickets (*Ceuthophilus*) hung from ceilings and walls, avoiding contact with pocket gophers. Earthworms were occasionally severed during excavation, but the wriggling remains of earthworm were treated with indifference. Ants (abundant in the surrounding trapping areas) were never found in burrow systems.

Pocket gophers showed little reaction to various amphibians. Likewise, *Ambystoma tigrinum* did not react to being walked on by pocket gophers while passing in the narrow tunnelway. In one instance, *Pappogeomys castanops* arrived

at the end of a tunnel, apparently to begin excavating, only to find a tiger salamander at rest. The pocket gopher picked the salamander up by the mid-dorsal skin with the incisors, turned, and deposited the animate obstruction 0.6 m away from the plug. The pocket gopher then returned to the plug to begin excavating. The salamander did not appear agitated or physically harmed. In another incident, a *Geomys bursarius* pushed a tiger salamander into a plug 5 consecutive times before the caudate was able to squeeze to one side allowing the persistent pocket gopher to work without further interference. Large *Bufo cognatus* were pushed aside or packed into plugs. A jumping toad would cause a pocket gopher to retreat backwards for a short distance before it returned to investigate further. *Scaphiopus couchi*, a smaller toad, were passively tolerated.

Lizards moved faster than amphibians in the burrow. An occasional pause by pocket gophers was the only reaction to the passing of the fast moving lizard (*Cnemidophorus sexlineatus*) in the tunnelway. Pocket gophers isolated the slower hog-nose snake, *Heterodon nasicus*, behind a dirt plug. After several min to several hrs, pocket gophers unplugged the restricted area for possible reclamation of the barricaded portion of the system. The same response was elicited by introducing a bull snake, *Pituophis melanoleucus*, into burrow systems. Dirt was excavated from the floor, walls, and ceiling of the burrow to block the advancing bull snake. Strikes by the snake appeared to bounce off the wall of incisors and foreclaws of the pocket gopher, and wall of dirt pushed at the snake.

*Perognathus flavus*, the silky pocket mouse, and *Onychomys leucogaster*, the grasshopper mouse, avoided pocket gophers with apparent ease. Nonetheless, both species never refused an opportunity to leave the burrow system. Larger rodents such as *Dipodomys spectablis*, the banner-tailed kangaroo rat, entered burrows of *Pappogeomys castanops* and *Geomys bursarius*, but not *Thomomys bottae*, because of the smaller tunnel diameter. Larger and slower mammals were herded to the surface or into a blind tunnel where retreat was impossible. Intruders were then sealed off with dirt. Kangaroo rats either dug a tunnel to the surface, or through the plug. However, one *Dipodomys spectablis* and a 13-lined ground squirrel died as a result of being buried alive. The 13-lined ground squirrels retreated as the pocket gopher "splashed" dirt in their direction.

Pocket gophers investigated any disturbance. If the pocket gopher acted dominant, the intruder was chased and any openings to the burrow plugged. In extreme cases, submissive pocket gophers would live on the surface. If equal, the resident or intruding pocket gopher quickly engaged in excavating dirt as a buffer between the pocket gopher and the disturbance. It was not unusual for 2 pocket gophers to add dirt to an intervening plug. Pocket gophers of large differential size could not interact within burrows because burrow walls restricted the movement of the large pocket gopher.

Interspecific interaction between adult pocket gophers favored the large pocket gopher (Table 1). Smaller pocket gophers intruding into larger burrows were invariably intimidated by the larger pocket gophers. Territorial behavior varied with the location of the encounter in the burrow system; for example, submissive pocket gophers became aggressive when trapped in a blind tunnel. Small residents were usually more submissive to larger pocket gophers in areas where the burrow diameter was dilated. Burrow diameters larger than normal may be caused by cave-ins, or by occupation of an old system. *Thomomys bottae*, being smaller than *Pappogeomys castanops* and *Geomys bursarius*, was at size disadvantage. In one instance, a 353 g *Pappogeomys castanops* trapped a small *Thomomys bottae* in a blind runway and killed the smaller pocket gopher by biting through the skull. Even a young *Pappogeomys castanops* was dominant over a mature *Thomomys bottae* because of size differences. Most encounters between pocket gophers were not mortal battles, usually ending instead with one pocket gopher retreating to the surface, or walling itself off in a small portion of the system.

## DISCUSSION

Pocket gophers may be afflicted by ectoparasites. English (1932) reported mites and lice, while Wilks (1963) did not find any external parasites on *G bursarius* (=*breviceps*) from Texas. No fleas or ticks were found on *Geomys, Pappogeomys,* of *Thomomys*.

Many non-parasitic forms of invertebrates have been first described from pocket gopher systems. Pseudoscorpions, dipterans, beetles, Collembolla, cave crickets, scorpions, spiders, centipedes, thysanurans, and moths (Ingles, 1952; Hermann, 1950; Hubbell and Goff, 1940) offer no serious obstruction to the activities of a pocket gopher (except as possible reservoirs for disease), or compete indirectly for habitat or food. The only inconvenience for a pocket gopher may be a bite from a large arthropod, whereby the pocket gopher may reciprocate by offering the temporary inconvenience of burial. Contact with ants are apparently avoided by rerouting of the tunnel system.

Vertebrates taken from geomyid burrow systems are surprisingly diverse. A frequently mentioned amphibian is the tiger salamander, *Ambystoma tigrinum* (Seton, 1929; Howard and Childs, 1959; Vaughan, 1961). Other amphibians found in the burrow systems of *Geomys bursarius* in Texas include *Bufo speciosus*, *B. valliceps, Scaphiopus couchi*, and *S. holbrooki* (Wilks, 1963). Amphibia pose no threat to the Geomyidae and vice-versa. Perhaps the poisonous epidermal glands of toads inhibits geomyids from biting or otherwise injuring amphibian intruders. A problem could arise for a pocket gopher if large numbers of anurans and caudates congested the tunnelways following a rain, but this has never been reported.

TABLE 1

Interaction of Pocket Gophers Released into the Observation Chamber (Intruders) with Pocket Gophers which Occupied an Established Burrow System (Residents). Three Weight Classes of Pocket Gopher were Tested 3 Times Each for Each of the 3 Species. An X Indicates no Interaction. In the Confrontations, the Resident was either Dominant (D), Equal (E), or Submissive (S) to the Intruder

| Weight Classes (g) | | *Thomomys bottae* (Resident) | | | *Geomys bursarius* (Resident) | | | *Pappogeomys castanops* (Resident) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 75–125 | 175–225 | 275–325 | 75–125 | 175–225 | 275–325 | 75–125 | 175–225 | 275–325 |
| *Thomomys bottae* (Intruder) | 75–125 | 87–94 D<br>75–88 D<br>98–94 E | X | X | 94– 75 D<br>98–105 D<br>87–118 D | 75–183 D<br>88–223 D<br>98–188 D | 94–288 D<br>84–279 D<br>85–293 D | 76– 95 D<br>85– 96 D<br>98–101 D | 88–191 D<br>84–218 D<br>94–222 D | 75–283 D<br>94–310 D<br>98–320 D |
| | 175–225 | X | X | X | X | X | X | X | X | X |
| | 275–325 | X | X | X | X | X | X | X | X | X |
| *Geomys bursarius* (Intruder) | 75–125 | 75–75 S<br>81–94 S<br>105–88 S | X | X | 75– 81 D<br>105– 93 E<br>81–105 D | 81–180 D<br>75–183 D<br>105–223 D | 75–271 D<br>105–284 D<br>81–278 D | 75– 95 E<br>105– 96 S<br>81– 95 E | 105–218 D<br>81–185 D<br>75–192 D | 105–283 D<br>81–310 D<br>75–320 D |
| | 175–225 | X | X | X | X | 180–183 D<br>223–178 E<br>183–223 D | 223–278 D<br>180–284 D<br>183–293 D | X | 180–218 D<br>223–192 E<br>178–222 D | 183–320 D<br>180–283 D<br>223–310 D |
| | 275–325 | X | X | X | X | X | 288–279 D<br>293–288 E<br>271–278 D | X | X | 271–283 E<br>293–310 D<br>278–320 D |
| *Pappogeomys castanops* (Intruder) | 75–125 | 95–98 S<br>96–94 S<br>95–88 E | X | X | 95–105 D<br>96–118 D<br>95–123 D | 95–223 D<br>95–183 D<br>96–188 D | 96–293 D<br>101–284 D<br>95–278 D | 95– 96 E<br>95– 95 E<br>96– 95 E | 96–218 D<br>101–222 D<br>95–192 D | 101–320 D<br>95–281 D<br>96–310 D |
| | 175–225 | X | X | X | X | 218–183 D<br>183–223 D<br>195–201 D | 183–284 D<br>218–278 D<br>222–288 D | X | 218–222 E<br>195–192 D<br>183–191 D | 183–320 D<br>222–310 D<br>195–283 D |
| | 275–325 | X | X | X | X | X | 310–293 D<br>320–284 D<br>281–288 D | X | X | 320–281 D<br>281–283 D<br>310–320 E |

Reptiles have been found in burrow systems. Lizards are likely only occasional visitors and are a little more than a nuisance when present. A whiptail lizard, *Cnemidophorus tigris*, has been found in a live-trap set for *Thomomys bottae* (Howard and Childs, 1959). The hognose snake, *Heterodon platyrhinus*, which has been taken from a burrow system of *Geomys bursarius* in Texas (Wilks, 1963), poses no threat to the adult pocket gopher, since *Heterodon* subsist exclusively on other reptiles and amphibians. Nonetheless, the pocket gopher is inconvenienced at least by a temporary loss of a portion of the tunnel system. The ability of the bull or gopher snake (*Pituophis melanoleucus*) to dig through the dirt-pushing defenses of a pocket gopher is one reason for this snake being a major predator of geomyids (Howard and Childs, 1959; Morse, 1927). The glossy snake (*Arizona elegans*), listed by Stebbins (1954) as a predator on pocket gophers, was found by Wilks (1963) in the burrow system of *Geomys bursarius*. Howard and Childs (1959) observed that *Thomomys bottae* did not retreat from a rattlesnake, but instead pushed dirt towards it. Snakes found in this study were restricted in striking ability because of limitations to coiling imposed by a narrow tunnelway.

An efficient mammalian predator entering geomyid burrow systems is the long-tailed weasel, *Mustela frenata* (Scheffer, 1945; Moore, 1945; Vaughan, 1961; Cummings, 1946). Most of the other carnivores such as bobcats, striped skunks, foxes, coyotes, badgers, and housecats known to prey on pocket gophers are too large to enter a geomyid burrow system. However, Scheffer (1910) recorded a spotted skunk trapped from a pocket gopher system.

Insectivores may infrequently intrude on geomyids. Scheffer (1945) found the mole, *Scalopus townsendii*, and the pocket gopher, *Thomomys talpoides*, caught in a runway constructed by the mole. Shrews may utilize the burrows of both moles and pocket gophers (Scheffer, 1954).

Rodents are frequently found in the burrows of pocket gophers. Howard and Childs (1959) live-trapped the deer mouse (*Peromyscus maniculatus*), pocket mouse (*Peromyscus inornatus*), and the kangaroo rat (*Dipodomys heermanni*) from the burrows of *Thomomys bottae*. Wilks (1963) thought that *Peromyscus hispidus* in burrows of *Geomys bursarius* in Texas robbed the food caches of the pocket gopher. *Perognathus* and the grasshopper mouse (*Onychomys leucogaster*) were similarly evasive in the present study, but left the burrow system given the opportunity. While Vaughan (1961) occasionally found ground squirrels forcing pocket gophers from pocket gopher burrow systems, Scheffer (1945) notes *Spermophilus townsendii* expelled from the burrow of *Thomomys talpoides*. *Spermophilus tridecemlineatus* might evict a smaller pocket gopher if the diameter of the burrow was dilated, but not under usual circumstances. The fact that geomyids lack the speed to apprehend smaller antagonists does not appear to be a serious handicap, since the ultimate goal of solitude is attained.

By plugging all openings to the surface, the pocket gopher not only excludes predators, but inhibits a host of interlopers seeking the safety and compatible environment of a burrow system (Vaughan, 1961). The small eyes and ears, short hair, large incisors which are permanently exposed, and large foreclaws all contribute to making the pocket gopher the dominant form of the subterranean environment in North America.Yet, the herbivorous dietary regimen of the Geomyidae precludes anything but a commensal or parasitic relationship with various arthropods and other moderate-sized fauna which benefit from the diversification of a subterranean habitat (Howard and Childs, 1959; Kennerly, 1964).

Questions as to which of the 3 North American genera of geomyids is most aggressive has been touched upon by Miller (1964) and Best (1973). Pocket gophers have the same physical appearance; differences are mainly quantitative, notably the proportionately shorter foreclaws of *Thomomys bottae* and the comparatively larger body sizes of *Geomys bursarius* and *Pappogeomys castanops*. A large pocket gopher has 2 advantages: the bite of the incisors, which is limited by the extreme length of the teeth, is proportionately larger and much more effective for a larger pocket gopher; and, a large pocket gopher can withstand wounds which would ordinarily prove fatal to a smaller pocket gopher. One *Pappogeomys castanops* used its large incisors to bite through the skull of one *Thomomys bottae*. Behavioural differences manifested in combat in the 3 species are few. Upon initial contact, pocket gophers assumed a threat posture with the anterior portion of the body slightly elevated, the mouth open, and one or both claws extended forward. Forward lunges with flailing claws and clashing incisors accompanied panting sounds. This commotion lasted less than a minute, when one animal would suddenly retreat. Deaths resulted only rarely. Soil acted as a buffer not only from surface environmental conditions, but also between the pocket gopher and intruders in the burrow system. If the intruder was not removed by force, a barrage of dirtloads isolated the disturbance. Normally, geomyid body size correlates with burrow diameter (Best, 1973) so that smaller pocket gophers are protected from geomyids too large to enter their burrows.

It appears from these studies that dominance is not measured only in terms of aggressiveness and success in combat. The competitiveness of interspecific and intraspecific interaction in geomyids may more importantly be determined by most efficient utilization of the habitat (Vaughan and Hansen, 1964; Reichman and Baker, 1972; Best, 1973).

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# MITOTIC CHROMOSOMES OF TURTLES. IV. THE EMYDIDAE

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ABSTRACT

Karyotypic data are presented on 30 species and subspecies of emydid turtles. Macrochromosome morphology and diploid numbers were compared to determine possible phylogenetic relationships. All New World (emydine) species had a diploid number of 50, while Old World (batagurine) species had 52 chromosomes. The largest 13 pairs of chromosomes were similar in morphology for all species examined. The major karyotypic difference noted was in the number of microchromosomes. The New World species had 12 pairs of microchromosomes, while the Old World species had 13 pairs.

The karyotypes of those emydids examined were also compared to those of several chelydrid and testudinid species. The macrochromosomes comprising the karyotypes of these 3 families were found to be morphologically similar. Comparison of diploid numbers indicates that testudinids, chelydrids, and Old World (batagurine) emydids are similar, while New World (emydine) emydids differ by possessing one fewer pair of microchromosomes. Thus, the karyotypic data reaffirms the karyotypic stability previously noted for cryptodiran turtles.

## INTRODUCTION

Chelonians, until recently, have been relatively neglected with respect to karyological research. Previously reported diploid numbers range from 26 to 66 (Ayres, *et al.*, 1969; Ohno, 1967) with increases in diploid number primarily attributable to increases in the number of microchromosomes.

The most common chromosome number for emydids is 50 (Huang and Clark, 1967; Matthey and van Brink, 1956; McKown, 1972; Stock, 1972). Emydids with a diploid number of 52, however, include the following: *Chinemys reevesii, Clemmys japonica* (Sasaki and Itoh, 1967), *Mauremys nigricans, Cyclemys dentata, Kachuga tecta* (Stock, 1972), *Graptemys barbouri* and *G. pulchra* (McKown, 1972). There are a number of emydids, however, which have not been karyotyped, or which have been incorrectly karyotyped. Thus, the purpose of this paper is to present additional karyotypic data for use in analyzing relationships among members of this and other closely related families of turtles.

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## MATERIALS AND METHODS

The method used for the study of emydid chromosomes is basically that of Gay and Kayfman (1950), with modifications for use on turtles (McKown, 1972). Karyotypes were prepared and examined from approximately 50 spreads per specimen. The chromosomes were classified according to Levan, *et al.,* (1964) and paired on the basis of size and the position of the centromere. Differences in size and morphology of the elements beyond the 13th pair were often difficult to ascertain; thus, only the largest 13 pair of chromosomes were considered as macrochromosomes (Killebrew, 1975).

Specimens were obtained primarily from pet dealers and by field collection of basking turtles using a long-handled dip net and a boat. All specimens used in this study are preserved and catalogued in the museum collection, Department of Biology, West Texas State University.

## RESULTS

Specimens of 30 species and subspecies, representing 15 emydid genera, were karyotyped (Table 1). All New World (emydine) genera had the same diploid number of 50, while all Old World (batagurine) genera had a diploid number of 52 (Table 1). The largest 13 pairs of chromosomes included 8 metacentric, 3 submetacentric, and 2 telocentric chromosome pairs in all specimens examined. The remaining 12 to 13 pairs were usually recognizable as metacentric or telocentric elements (Fig. 1).

The 50 chromosome karyotype reported for *Ocadia sinensis* and *Siebenrockiella crassicollis* (Stock, 1972), and the diploid number of 52 for *Graptemys barbouri* and *G. pulchra* (McKown, 1972) are not supported by this study. The 52 chromosome karyotype presented for *Ocadia sinensis* is similar to that reported by Nakamura (1949).

## DISCUSSION

Data on emydid karyotypes presented in this study may be taxonomically important. Comparison on diploid numbers separates emydids into New World (emydine) genera (2n = 50), including *Emys orbicularis* (van Brink, 1959), and Old World (batagurine) genera (2n = 52), including *Rhinoclemys.* Analysis of the macrochromosome morphology, however, reveals that all emydids are karyotypically similar. Thus, the karyotypic datum supports the division of the Emydidae into 2 subfamilies, the Emydinae and the Batagurinae (McDowell, 1964).

Comparison of emydid (present study) and testudinid (Ohno, 1967; Sampaio, *et al.,* 1971; Stock, 1972) karyotypes reveals a similarity between the diploid number (2n = 52) and morphology of batagurine and testudinid karyotypes. Emydine genera (present study) are also similar to testudinids in the morphology of

TABLE 1

Diploid number and collection source for those species of emydids examined. Abbreviations in the table represent the following: M = macrochromosomes, m = microchromosomes, 2n = diploid number.

| Species | Diploid Number M | m | 2n | Source |
|---|---|---|---|---|
| *Chinemys reevesii* | 26 | 26 | 52 | Midwest Reptile |
| *Chrysemys picta* | 26 | 24 | 50 | R. McKown |
| *Clemmys caspica leprosa* | 26 | 24 | 50 | Midwest Reptile |
| *Clemmys marmorata pallida* | 26 | 24 | 50 | R. McKown |
| *Cuora amboinensis* | 26 | 26 | 52 | R. McKown |
| *Cyclemys dentata* | 26 | 26 | 52 | Midwest Reptile |
| *Deirochelys reticularia reticularia* | 26 | 24 | 50 | Midwest Reptile |
| *Graptemys caglei* | 26 | 24 | 50 | Guadalupe River |
| *Graptemys barbouri* | 26 | 24 | 50 | Chipola River |
| *Graptemys flavimaculata* | 26 | 24 | 50 | Pascagoula River |
| *Graptemys geographica* | 26 | 24 | 50 | Buffalo River |
| *Graptemys kohni* | 26 | 24 | 50 | Derl Brooks |
| *Graptemys nigrinoda* | 26 | 24 | 50 | Alabama River |
| *Graptemys oculifera* | 26 | 24 | 50 | Pearl River |
| *Graptemys pseudogeographica sabinensis* | 26 | 24 | 50 | Sabine River |
| *Kachuga smithi* | 26 | 26 | 52 | Midwest Reptile |
| *Mauremys mutica* | 26 | 26 | 52 | Midwest Reptile |
| *Ocadia sinensis* | 26 | 26 | 52 | Midwest Reptile |
| *Pseudemys concinna texana* | 26 | 24 | 50 | Guadalupe River |
| *Pseudemys floridana hoyi* | 26 | 24 | 50 | Sabine River |
| *Pseudemys scripta callirostris* | 26 | 24 | 50 | Moses Department Store |
| *Pseudemys scripta elegans* | 26 | 24 | 50 | Sabine River |
| *Pseudemys nelsoni* | 26 | 24 | 50 | Midwest Reptile |
| *Malayemys subtrijuga* | 26 | 26 | 52 | Midwest Reptile |
| *Rhinoclemys funeria* | 26 | 26 | 52 | Midwest Reptile |
| *Rhinoclemys punctularia melanosterna* | 26 | 26 | 52 | F. Medem |
| *Siebenrockiella crassicollis* | 26 | 26 | 52 | Midwest Reptile |
| *Terrapene carolina triunguis* | 26 | 24 | 50 | Pike Co., Arkansas |
| *Terrapene coahuila* | 26 | 24 | 50 | Cuatro Cienegas, Mexico |
| *Terrapene ornata* | 26 | 24 | 50 | Randall Co., Texas |

the macrochromosomes but differ by possessing one fewer pair of microchromosomes. Thus, the close relationship between emydid and testudinid genera is supported by the karyotypic data. This is interesting since ancestral emydids are thought to be of Asiatic origin (Loveridge and Williams, 1957). If one assumes that these

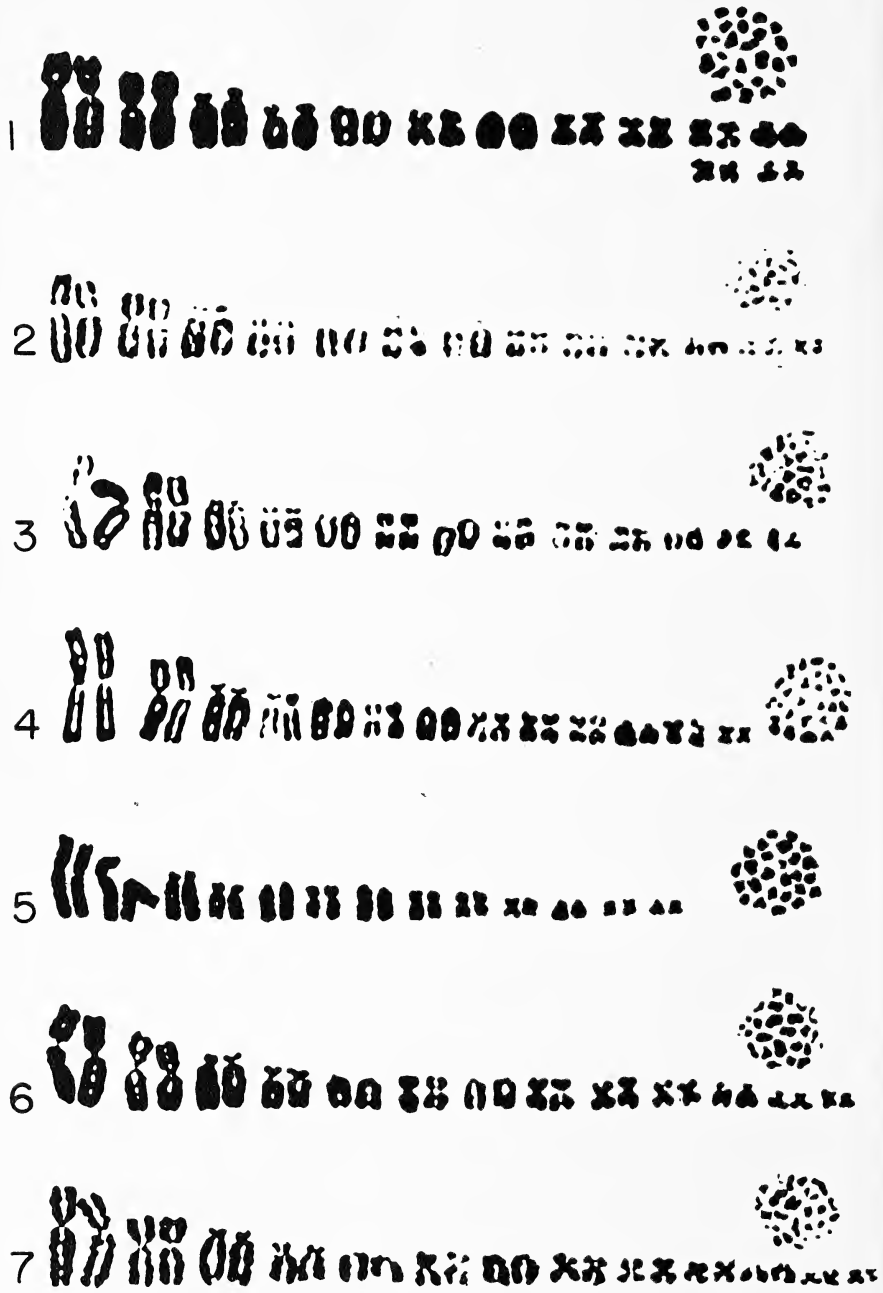


Figure 1. Karyotypes are as follows: 1. *Chinemys reevesii*, 2n = 52; 2. *Chrysemys picta*, 2n = 50; 3. *Clemmys caspica leprosa*, 2n = 50; 4. *Clemmys marmorata pallida*, 2n = 50; 5. *Cuora amboinensis*, 2n = 52; 6. *Cyclemys dentata*, 2n = 52; 7. *Deirochelys reticularia reticularia*, 2n = 50.

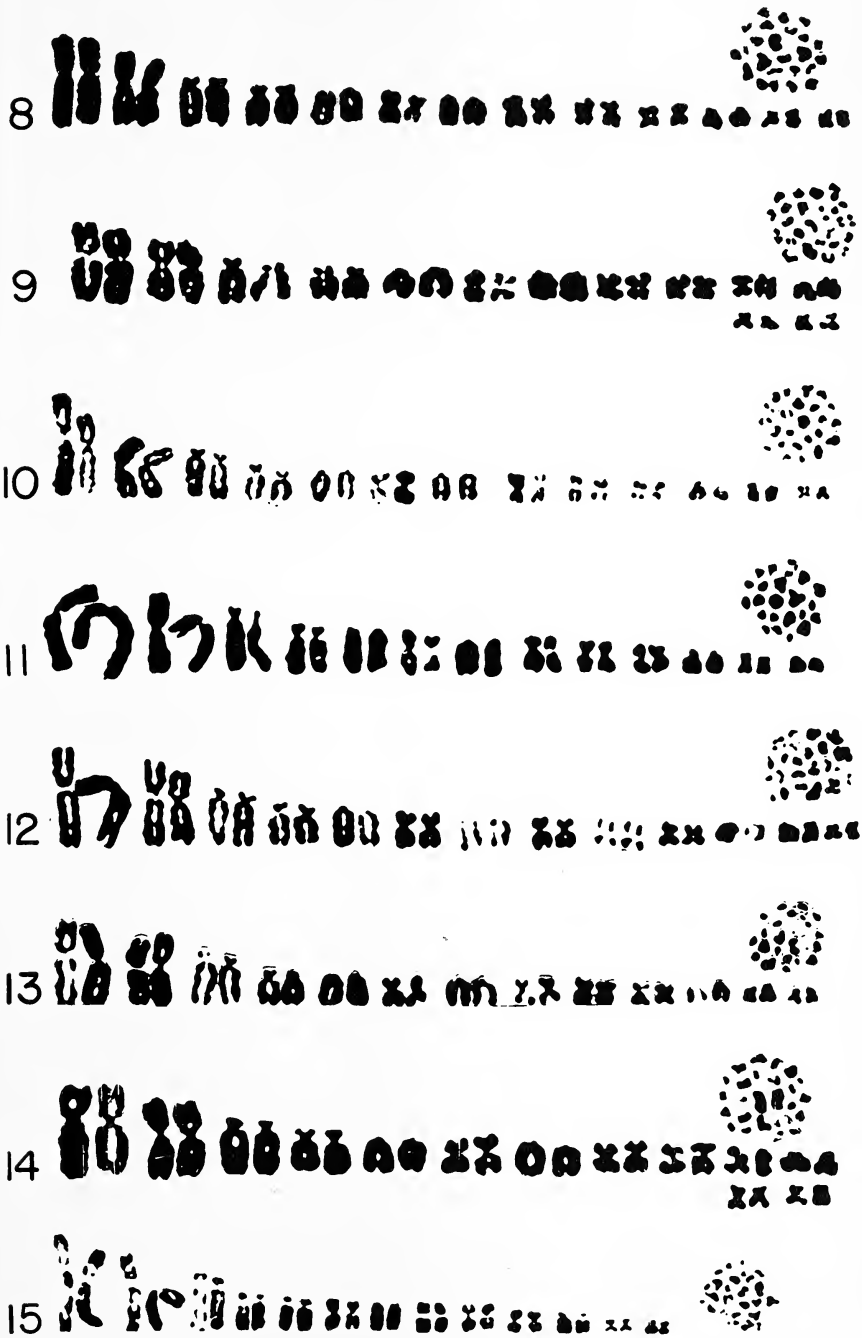


Figure 1. (Continued) 8. *Graptemys caglei*, 2n = 50; 9. *Graptemys barbouri*, 2n = 50; 10. *Graptemys flavimaculata*, 2n = 50; 11. *Graptemys geographica*, 2n = 50; 12. *Graptemys kohni*, 2n = 50; 13. *Graptemys nigrinoda*, 2n = 50; 14. *Graptemys oculifera*, 2n = 50; 15. *Graptemys pseudogeographica sabinensis*, 2n = 50.

Figure 1. (Continued) 16. *Kachuga smithi,* 2n = 52; 17. *Mauremys mutica,* 2n = 52; 18. *Ocadia sinensis,* 2n = 52; 19. *Pseudemys concinna texana,* 2n = 50; 20. *Pseudemys floridana hoyi,* 2n = 50; 21. *Pseudemys scripta callirostris,* 2n = 50; 22. *Pseudemys scripta elegans,* 2n = 50; 23. *Pseudemys nelsoni,* 2n = 50.

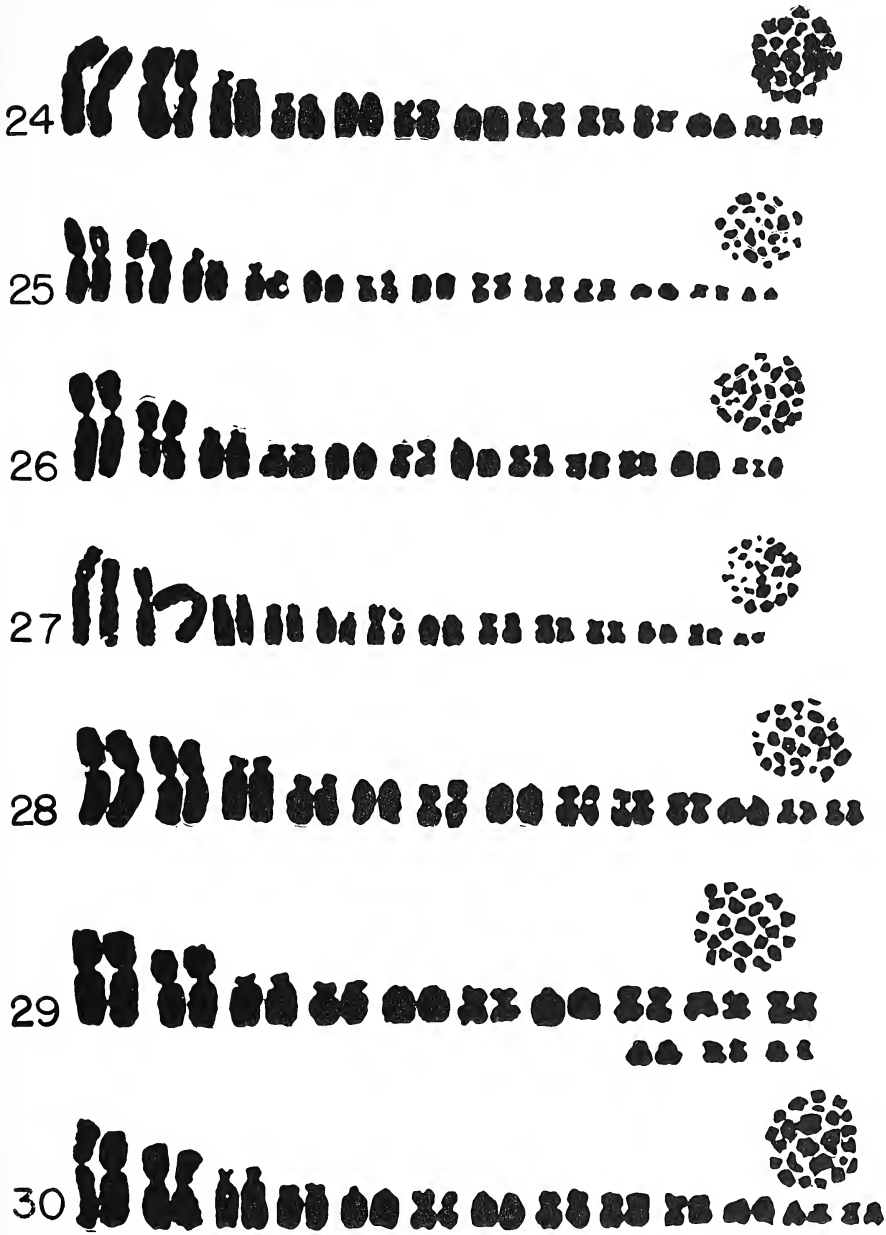


Figure 1. (Continued) 24. *Malayemys subtrijuga,* 2n = 52; 25. *Rhinoclemys funeria,* 2n = 52; 26. *Rhinoclemys punctularia melanosterna,* 2n = 52; 27. *Siebenrockiella crassicollis,* 2n = 52; 28. *Terrapene carolina,* 2n = 50; 29. *Terrapene coahuila,* 2n = 50; 30. *Terrapene ornata,* 2n = 50.

ancestral emydids had a 52 chromosome karyotype, as do those present-day Asiatic genera that were examined, then derivation of the 50 chromosome emydid karyotype would require either the deletion of 1 pair of microchromosomes or the fusion of 2 pair of these elements. If one assumes, however, that the ancestral emydids had 50 rather than 52 chromosomes, then some other mechanism, such as chromosomal breakage, would be required to explain the extra pair of batagurine microchromosomes. Present analyses of microchromosome morphology, however, are not sufficient to resolve this question. Another alternative is that the ancestral emydids had a diploid number other than 50 or 52. However, this is not supported by available evidence.

The emydid karyotypes presented are also very similar to those of chelydrids (Stock, 1972). Chelydrids, until recently, were thought to be more closely related to the kinosternids (Williams, 1950) than to the emydids. Recent studies of serum proteins (Friar, 1962, 1964, 1972), however, indicate that the chelydrids are closer to the New World emydids than to kinosternids. Analysis of the morphology of the macrochromosomes of these groups indicates that the chelydrids, emydids, and staurotypinės are karyotypically similar, while kinosternines are more divergent (Killebrew, *In Press*; Stock, 1972), and the second pair of macrochromosomes is submetacentric in the kinosternines and metacentric in the chelydrids, emydids, and staurotypines.

A different grouping is obtained, however, when diploid numbers are compared. According to this grouping, New World emydids (2n = 50) form 1 group, chelydrids and Old World emydids (2n = 52) form a 2nd group, staurotypines (2n = 54) form a 3rd group, and kinosternines (2n = 56) form a 4th group. Thus, a grouping based on diploid number supports the theory that the chelydrids are more closely related to emydids than to the kinosternids. The karyotypic evidence also indicates that the chelydrids share karyotypic affinities with the kinosternids, since staurotypines have a morphologically similar karyotype and a diploid number that differs from the chelydrid karyotype by one pair of microchromosomes. Thus, the karyotypic data supports the contention that the kinosternids and chelydrids arose from a common stock which is close to the emydids.

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# EFFECTS OF HYPOPHYSECTOMY IN THE LIZARD *HOLBROOKIA PROPINQUA*

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ABSTRACT

With more interest in comparative studies of various interests, such as reproduction, the importance of the phylogenetic position of the reptiles is once again pointed out. Although relatively ignored, the reptiles are being subjected to an increasing scrutiny in the laboratory, but even more work is needed to get the reptilian "state of the art" to levels comparable with the other vertebrate classes. This investigation provides additional information on yet another species.

## INTRODUCTION

In this investigation the effects of hypophysectomy on the testis and spermatogenesis were studied in the lizard *Holbrookia propinqua*. Secondary effects, such as those concerning the androgen-sensitive epithelial tissues of the epididymus and renal sex segment, as well as fat body weight, which shows cyclicity coincident with reproductive cycles, were also studied. This study provides quantification of some effects of hypophysectomy in this species by considering the relationship of time and the appearance of these effects.

## MATERIALS AND METHODS

The animals used were keeled, earless lizards, *Holbrookia propinqua*, collected on Padre Island[1] off the Texas coast during July and August, 1973. The experiments were begun after the animals had been held 2 wks. The lizards were housed in plywood cages containing several cm of sand. The cage floors measured approximately 60 to 70 cm and 20 to 30 lizards were kept in a cage. Photothermal requirements were supplied by a bank of lights, controlled by a timer, providing 14 hrs of light and 10 hrs of darkness. The lights were 75 watt flood lights directed toward the floor of the cage about 15 cm from one side. This provided a temperature gradient across the cage floor. Maximum temperature was main-

[1]Collecting was done on the Padre Island National Seashore with permission of the U.S. National Park Service.

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tained at 35–37 C, with the lights on, falling to 20–24 C when the lights were off. Some animals did not feed well in the laboratory, so the entire group was force-fed meal worms to maintain a constant nutritional state.

In *H. propinqua* the pituitary is visible in the roof of the mouth and consequently can be removed with relative ease intraorally. A small hole was made in the cartilage just below the gland with a dental burr, size #1/2-round. The pituitary was then aspirated through this hole with a blunt 20 gauge needle attached to a vacuum source and no further treatment was necessary.

Testes, epididymi, kidneys, and heads were fixed in Bouins' fluid, embedded in paraffin and sectioned at 7 $\mu$m. Staining was done using Harris' hematoxylin and an eosin-phloxine counterstain. The heads were sectioned serially at 10 $\mu$m to confirm the completeness of hypophysectomy and where pituitary tissue was identified the data were not used.

At autopsy, total body weight was recorded as well as weights of the testes and fat bodies. Measurements of epithelial cell heights were made randomly using a graticule scale with 5 measurements recorded of each tissue for each animal. Means and standard errors were calculated and t-tests performed on the mensural data.

This study was designed, in part, to determine the time needed for responses to pituitary ablation to occur. Two groups of animals were hypophysectomized and examined at various intervals to determine the effects. The first group was autopsied over a longer time base which gave an overview of the temporal relationships. This suggested the second group study, which produced additional data, expanding the first half of the original study. The first group was autopsied in subgroups at 0, 5, 10, 15 and 20 days and the second group at 0, 3, 5, 7 and 10 days.

## RESULTS

Results of these studies are presented in Tables 1 and 2. The data show a pattern of testicular regression, and following closely, a similar decline in tissues that are dependent upon androgen stimulation. Time periods required for the effects of endocrine ablation to appear on reproductive structures are shown.

In the 20-day study, total body weight dropped, inexplicably, in both the control and hypophysectomized groups at about day 10. This weight loss appeared in the organ weights and measurements for the control group but how much it is reflected by the hypophysectomy data is not as clear. With the exception of epididymal epithelial height, at day 10, the other measurements gradually decreased (except for the increase in fat body weight).

Testes (Fig. 1) began to lose weight between days 3 and 5 after hypophysectomy. Testis weight showed similar values in the 2 hypophysectomized groups and was significantly reduced by day 10 in both studies. Weight loss continued until approximately day 15, when the testes reached their minimum weight. The

TABLE 1

Hypophysectomy Effects – Males – 20 Days

| Days After Hypophysectomy | | 0 | 3 | 5 | 7 | 10 |
|---|---|---|---|---|---|---|
| Body Weight (g) | Control | 3.50 ± 0.308[1] | | 3.07 ± 0.295 | | 3.92 ± 0.378 |
| | Hypox | | 3.29 ± 0.138 | 3.20 ± 0.208 | 3.58 ± 0.291 | 3.72 ± 0.222 |
| Testis Weight (mg) | Control | 33.8 ± 4.60 | | 16.3 ± 4.08 | | 35.5 ± 5.56 |
| | Hypox | | 12.0 ± 2.08 | 7.1 ± 2.40*[2] | 3.3 ± 0.29 | 3.4 ± 0.69*** |
| Epidiymal Epithelium Height (μm) | Control | 28.6 ± 1.01 | | 21.3 ± 1.04 | | 26.2 ± 1.71 |
| | Hypox | | 20.5 ± 0.75 | 15.4 ± 0.58*** | 13.8 ± 0.45 | 16.2 ± 1.15*** |
| Renal Sex Segment Epithelium Height (μm) | Control | 29.3 ± 0.77 | | 22.9 ± 0.78 | | 28.8 ± 1.20 |
| | Hypox | | 24.0 ± 0.64 | 17.0 ± 0.61*** | 13.8 ± 0.45 | 16.2 ± 1.15 |
| Fat Body Weight (mg) | Control | 8.4 ± 3.12 | | 7.3 ± 3.37 | | 10.9 ± 4.13 |
| | Hypox | | 5.4 ± 1.08 | 7.5 ± 1.07 | 14.7 ± 4.78 | 30.4 ± 4.13** |
| Animals /Group | Control | 10 | | 7 | | 8 |
| | Hypox | | 8 | 9 | 9 | 7 |

[1] Mean ± Standard Error.
[2] * .05; ** .005; *** .001

TABLE 2

Hypophysectomy Effects – Males – 10 Days

| Days After Hypophysectomy | | 0 | 3 | 5 | 7 | 10 |
|---|---|---|---|---|---|---|
| Body Weight (g) | Control | 3.64 ± 0.195[1] | 3.77 ± 0.164 | 3.67 ± 0.132 | 4.08 ± 0.174 | 3.32 ± 0.134 |
| | Hypox | | 4.04 ± 0.203 | 3.72 ± 0.206 | 3.31 ± 0.074 | 3.40 ± 0.223 |
| Testis Weight (mg) | Control | 36.0 ± 4.45 | 38.1 ± 2.97 | 34.0 ± 4.22 | 39.2 ± 3.70 | 26.0 ± 3.24 |
| | Hypox | | 44.5 ± 2.32 | 22.2 ± 3.07* | 14.9 ± 1.97**** | 7.5 ± 1.05**** |
| Epididymal Epithelium Height (μm) | Control | 30.9 ± 0.94 | 32.8 ± 0.64 | 39.2 ± 1.02 | 30.4 ± 0.58 | 28.2 ± 1.02 |
| | Hypox | | 31.0 ± 0.64 | 28.3 ± 0.70**** | 22.1 ± 0.62**** | 17.1 ± 0.59**** |
| Renal Sex Segment Epithelium Height (μm) | Control | 26.6 ± 0.58 | 26.7 ± 0.54 | 27.4 ± 0.61 | 28.3 ± 0.51 | 24.3 ± 0.51 |
| | Hypox | | 27.4 ± 0.50 | 23.4 ± 0.54**** | 24.5 ± 0.58**** | 22.4 ± 0.43 |
| Fat Body Weight (mg) | Control | 9.4 ± 2.69 | 13.2 ± 3.23 | 11.9 ± 3.20 | 13.1 ± 1.98 | 8.3 ± 3.52 |
| | Hypox | | 21.0 ± 2.30 | 12.7 ± 2.30 | 12.1 ± 2.16 | 13.6 ± 1.84 |
| Animals/Group | Control | 10 | 10 | 10 | 10 | 11 |
| | Hypox | | 10 | 8 | 9 | 10 |

[1]Mean ± Standard Error.
[2]*.05; **.01; ***.005; ****.001

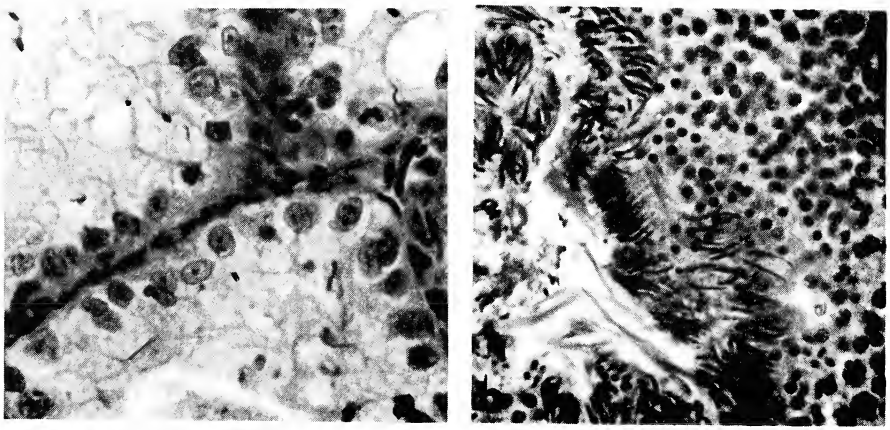

Figure 1. Effects of hypophysectomy on the testis. A is from 20 day post-hypophysectomy, B is control. Note reduction in seminiferous tubule size and loss of germinal epithelium. 500X

spermatic stage, a system of ranking spermatic activity (Table 3) showed a similar rate of involution, seen as a loss of organization of the germinal epithelium and lack of sperm in the epididymus, occurring between days 3 and 5 with complete disorganization occurring by day 10 (Fig. 4).

TABLE 3

Spermatic Stage Criteria[1]

| Stage | Histological Condition | |
|---|---|---|
| | Seminiferous Tubules | Epididymus |
| 0 | Involuted with epithelium in disarray | Atrophic, empty |
| 1 | Involuted with only spermatogonia | Atrophic, empty |
| 2 | Primary spermatogonia appearing | Atrophic, empty |
| 3 | Secondary spermatocytes and early spermatids | Atrophic, empty |
| 4 | Transforming spermatids with a few spermatozoa | Atrophic, empty |
| 5 | Spermatids and spermatozoa abundant | Hypertrophied, empty |
| 6 | Spermatozoa abundant and spermatocytes reduced | Hypertrophied with many sperm |

[1]Modified from Licht, 1967.

The effects of hypophysectomy on the androgen-maintained epithelium of the epididymis and the renal sex segment appeared at day 5 or soon after. Cell height reached minimum values in the epididymi by day 10 and appeared to do

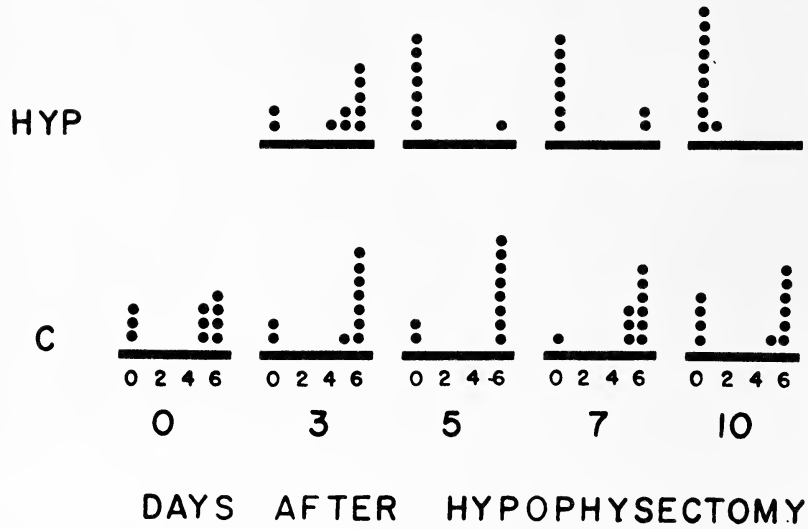


Figure 4. Effects of hypophysectomy on spermatic stage. Each dot represents the placement of 1 animal with regard to the criteria numbered 0 through 6 in Table 3. Notice the control group (c) values tend to occur most often at 5 and 6 with a few at the low end. The treated group (Hyp) shows the same pattern at day 3 as the controls but from day 5 on shows the reverse, suggesting the effects on the germinal epithelium are manifested before day 5.

so in the sex segment at or soon after that time (Figs. 2 and 3). Fat body weight did not change as rapidly as the other parameters but increased by day 20 after hypophysectomy.

## DISCUSSION

Effects of hypophysectomy on reproduction in reptiles has been described primarily as gonadal involution. This is accompanied by regression of tissues that are dependent upon steroid secretion by the gonad (Cieslak, 1945; Wagner, 1955; Eyeson, 1971; Tsui and Licht, 1974). From the data presented in this study, it can be seen that hypophysectomy in *Holbrookia propinqua* produces the same general effects on the reproductive system that occur in other reptiles. Further, it is possible to make some statement on the time element needed to manifest these effects.

Minimum time required to see the effects of gonadotropin depletion was approximately 3 days. Panda and Thapliyal (1964) reported that 13 days were necessary for complete regression of the germinal tissues of testes in the lizard *Calotes versicolor* and pointed out the contrast to the 9 days necessary in snakes reported by Cieslak (1945). Data presented here show considerable reduction of the germ-

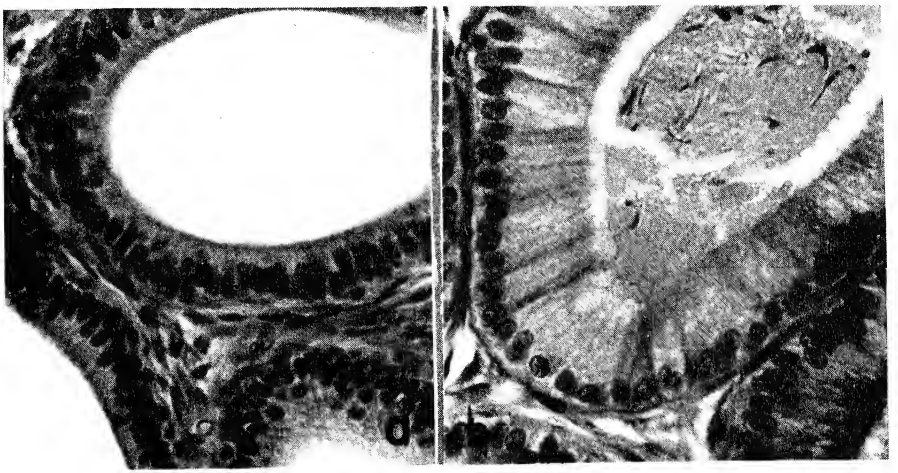

Figure 2. Effects of hypophysectomy on the epididymus. The epithelium is much reduced and the tubules are empty at 20 days after operation. A is from 20 day post-hypophysectomy, B is control. 500X

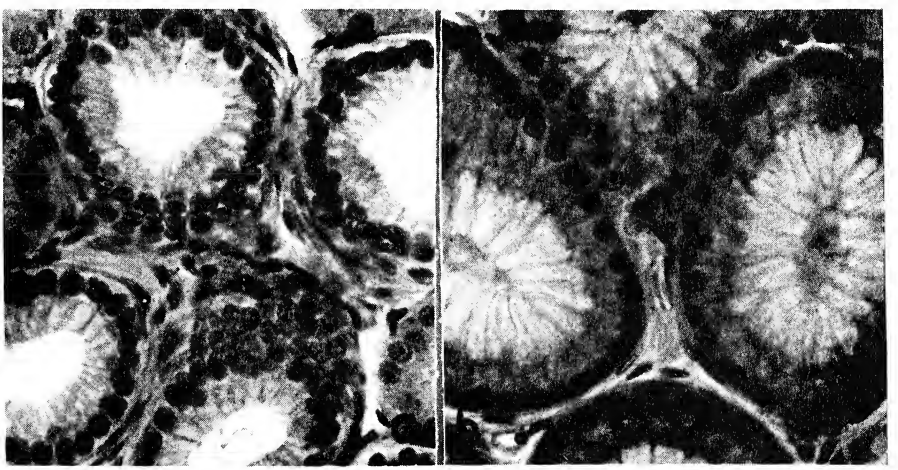

Figure 3. Effects of hypophysectomy on the renal sex segment. There is loss of secretory material in the cells and reduction in cell height. A is from 20 day post-hypophysectomy, B is control. 500X

inal epithelium (described as spermatic stages) beginning between 3 and 5 days after hypophysectomy with complete involution at or soon after day 10.

Rapid regression in testicular size and spermatogenic activity after hypophysectomy was reported in the lizard *Anolis carolinensis* when the animals were kept at 31 C. Only slight changes occurred in the testes of animals kept at 20 C.

Short daylengths at 31 C produced the same testicular effects as hypophysectomy (Licht and Pearson, 1969). In this study, temperature was maintained above 31 C at temperatures nearer those of the natural habitat and photoperiod was 14 hrs light, 10 hrs dark which also approximates natural conditions.

Effects of hypophysectomy on the interstitial tissue are shown indirectly as reductions of androgen-sensitive epithelial tissues. These tissues, the epididymis and renal sex segment, regressed to minimal values at day 10 following hypophysectomy. Ignoring any time needed to deplete circulating hormones (i.e. temporal differences between cessation of androgen secretion and involution of androgen dependent tissues), one could speculate that the interstitial tissue had involuted within 10 days after hypophysectomy.

In this study, as reproductive tissues regressed to conditions compatible with those of non-reproductive lizards, the fat bodies began to increase in weight. These changes are consistent with reports that fat body cycles correlate with yearly reproductive cycles where fat body weights are at minimal values during periods of gonadal activity (Dessauer and Fox, 1959; Hahn and Tinkle, 1966).

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# CRETACEOUS (ALBIAN) AMMONITES FROM PUERTO RICO AND ST. THOMAS

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ABSTRACT

Except for Cuba, ammonites from the Caribbean Islands have been less than abundant, although a few have been described from Jamaica, Curacao, Haiti, and Trinidad. Herein are described Albian ammonites from St. Thomas of the Virgin Islands and from Puerto Rico. *Manuaniceras* sp. cf. *M. supani* (Lasswitz) is from the Middle Albian Robles Formation of the Comerio Quadrangle, Puerto Rico. *Eogaudryceras* (?) sp., *Puzosia* sp. cf. *P. welwitschi* Choffat and de Loriol, and *Engonoceras* spp. are from the pre-Robles beds, but also are most probably Middle Albian.

*Mortoniceras* sp. juv. cf. *M. scobinum* (Van Hoepen), *M.* sp. juv. cf. *M. nanum* Spath, and *Hypophylloceras* sp. juv. cf. *H. ellipticum* (Kossmat) are from the Upper Albian part of the Tutu Formation, west end of St. Thomas, Virgin Islands.

## INTRODUCTION

Very few ammonites have been described from the Caribbean Islands, except for Cuba. Spath (1925a) described Campanian ammonites from Jamaica, and Matsumoto (1966) described a Cretaceous ammonite from Curacao. Reeside (1947) described a few Lower Senonian ammonites from Haiti, and Spath (1939) described latest Jurassic ammonites from Trinidad. Cuban ammonites are largely late Jurassic (Imlay, 1942; O'Connell, 1920). Meyorhoff (1933) mentions Middle Cretaceous ammonites from Puerto Rico, identified by J.B. Reeside, Jr. Still another ammonite from Puerto Rico was identified as belonging to the genus *Manuaniceras*, perhaps *M. carbonarium*, by W.A. Cobban (Sohl, 1975); it was collected from the Fajardo Shales at the northeast end of the island. Although many Cretaceous formations can be assigned to stages by rudists and foraminiferans, still, corroborative evidence is always desirable. In areas of good ammonite collecting the forms described herein would not merit a paper. Since they constitute all but 2 of the ammonites known from the islands of St. Thomas and Puerto Rico, their importance is emphasized by their uniqueness.

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*Ammonites From Puerto Rico*

Early in the 1960's I received from Norman Sohl 2 ammonites from the Cretaceous of Puerto Rico. One was an oxytropidoceroid of which the Middle Albian age was never in doubt. The second was a puzosiid, which I compared to *Austiniceras dibleyi* Spath (1922) and considered either Turonian or Coniacian. Further stratigraphic work in Puerto Rico indicated that the horizon of the puzosiid was older than that of the oxytropidoceroid, which was Middle Albian; the stratigraphers correctly followed the dictates of superposition from field work and ignored my identification.

In 1968 Erle Kauffman, Norman Sohl, Bob Perkins, and I revisited this locality (U.S. Geological Survey Mesozoic Locality 26087 = U.S. Geological Survey Mesozoic Locality 26804) and collected more specimens of the puzosiid, plus specimens of engonocerids and *Eogaudryceras* (?) sp. Upon seeing the eogaudrycerine I changed my identification of the puzosiid at the site. I now consider that meager and poorly preserved fauna to consist of the following forms:

*Puzosia* sp. cf. *P. welwitschi* Choffat and de Loriol, 1888.

*Eogaudryceras* (?) sp.

*Engonoceras* spp. indet.

The rock containing the fossils is a dirty sandstone, somewhat clayey, that appears to be composed of fragments reworked largely from mafic bentonites. It is an environment in which ammonites are seldom found. The coarse, granular, clayey rocks preserve no shell material, and the internal molds are devoid of sutures, growth lines, and most other ornamentation. A few faint ribs appear on some specimens. Under such circumstances of preservation all identifications are precarious and suspect.

In 1972 I suggested that this fauna was Lower Albian. This was on the basis of work by stratigraphers indicating that the containing beds underlay undoubted Middle Albian, and also on the identification of *Eogaudryceras* sp. Further study has shown that the Puerto Rican *Eogaudryceras* (?) cannot be demonstrated to be Lower Albian. Furthermore, the genus *Puzosia* s.s. is not supposed to extend below the Middle Albian, which is the most likely age of *Puzosia welwitschi* Choffat and de Loriol (1888) and *P. cuvervillei* (Meunier, 1888), the 2 species to which the Puerto Rican puzosiid is most easily compared. This places a Middle Albian date on this locality of the pre-Robles beds, some 3 mi north of Aiboneto, Barranquitas quadrangle, Puerto Rico.

The previously mentioned oxytropidoceroid is from the Robles Formation, about the middle of a very thick sequence. It is a Middle Albian *Manuaniceras*, probably *M. supani* (Lasswitz, 1904), well known in the Texas Comanche Peak and Goodland Formations. The ribbing is that of older *M. supani*, rather than younger *supani*, and indicates an age high in the Middle Albian, according to zonations of Breistroffer (1947), and Young (1966). Thus we have an undoubted Middle Albian fauna from the Robles Formation (Berryhill, *et al.*, 1960; Donnelly, 1970a), overlying by as much as 300 or more meters of rock an indicated Middle Albian fauna (pre-Robles beds), but in different areas.

*St. Thomas Ammonites*

The 3 micromorphs (Plate 2, Figs 19-24), all silicified, from the Tutu Formation (Donnelly, 1970b), western end of the island of St. Thomas, Virgin Islands, are all juveniles. The specimen of *Hypophylloceras*, herein compared with *H.* sp. group of *H. ellipticum* (Kossmat), is too generalized and too juvenile to identify with precision. The 2 juvenile specimens of the genus *Mortoniceras* are likewise too small to identify specifically, but small specimens of this genus, particularly the binodose forms with ribs bifurcating at the umbilical node, are so unique they can be dated as Upper Albian.

## SYSTEMATIC DESCRIPTIONS

Phylum Mollusca
Class Cephalopoda
Order Ammonoidea
Suborder Phylloceratina Arkell, 1950
Superfamily Phylloceratacea Zittel, 1884
Family Phylloceratida Zittel, 1884
Subfamily Phylloceratinae Zittel, 1884
Genus *Hypophylloceras* Salfeld, 1924

*Hypophylloceras* sp. cf. group of *H. ellipticum*
(Kossmat, 1895)
Pl. 2, Figs. 23, 24

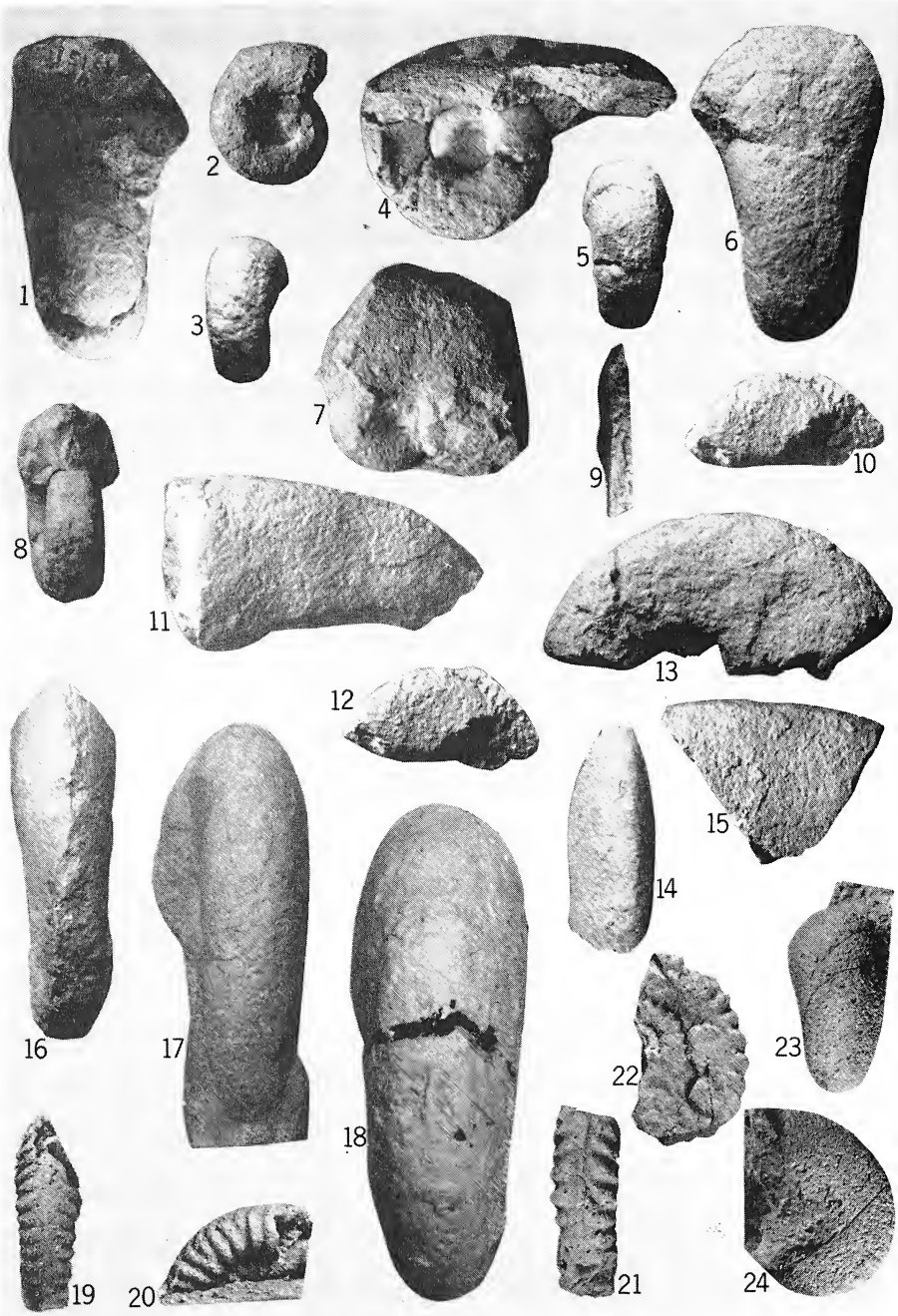
=*Hypophylloceras* sp. juv. cf. *H. velledae* (Michelin) in Young, 1972, p. 469.
*cf. Phylloceras ellipticum* Kossmat, 1895, Pl. 6, Figs. 1ab

*Dimensions.*

| D | H | W | U | H/W |
|---|---|---|---|---|
| 16.0 | 70.5 | 53.0 | 6.7 | 1.33 |

*Remarks.* The specimen illustrated on Pl. 2, Figs. 23 and 24 is about the same size as the pyritic micromorphs illustrated by Pervinquière [1910, Pl. 1 (10)], but is much less well preserved. This makes it impossible to identify, although, because it has a more rapidly expanding whorl than those illustrated by Pervinquiere, it would appear to be more closely related to *H. ellipticum* (Kossmat,1895, Pl. 6, Figs. 1ab) and *H. subalpinum* (d'Orbigny, 1850 = *Ammonites alpinus* d'Orbigny, 1840-42, p 283, Pl. 83). The latter species was discussed by Fallot (1910) and Gignoux (1920), and was illustrated by Spath (1923, Pl. 1, Figs. 1ab, 2) and Wiedmann (1962a, Pl. 16, Figs. 2ab). The St. Thomas specimen has the greater width of the whorl more ventrad than does *H. subalpinus* (d'Orbigny), and in this way also resembles *H. ellipticum* (Kossmat). *H. cypris* (Fallot and Termier, 1923, Pl. 3, Figs. 1 and 2) is less involute than the St. Thomas specimen.

The St. Thomas specimen is, then, unlike those specimens Wiedmann (1962a, p 254) groups together as related to *H. onoense* (Stanton, 1895), but is very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## DESCRIPTION OF PLATE 2

1–8 *Eogaudryceras* ? sp. 1–6, 8, ventral and lateral views of WSA 16307; 7, WSA 16301; both from pre–Robles beds about 3 mi north of Aiboneto, Puerto Rico. U.S.G.S. Mesozoic locality 26087.

9–12, 14, 15 *Engonoceras* ? spp. 9, 10, 12, WSA 16298; 11. WSA 16300 14, 15, WSA 16299; all from pre–Robles formation beds about 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087.

13, 16–18 *Puzosia* sp. cf. *P. welwitschi* Choffat and de Loriol, 1888, 13, 16, lateral and ventral views of WSA 16300, 17, ventral view of WSA 16302 (see also Pl. 1, Figs. 7–9); 18, ventral view of WSA 16306; all from pre–Robles beds, 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087.

19–20 *Mortoniceras* sp. juv. cf. *M. nanum* Spath, ventral and lateral views of specimen at Syracuse University from the Tutu Formation, west end of St. Thomas, Virgin Islands, collected by T.W. Donnelly.

21–22 *Mortoniceras* sp. juv. cf. *M. scobinum* (Van Hoepen), lateral and ventral views of a specimen at Syracuse University from the Tutu Formation, west end of St. Thomas, Virgin Islands, collected by T.W. Donnelly.

23–24 *Hypophylloceras* sp. cf. *ellipticum* (Kossmat), ventral and lateral views of a specimen at Syracuse University from the Tutu Formation west end of St. Thomas, Virgin Islands, collected by T.W. Donnelly.
1–16, 18, X 1; 17, X 0.5; 19–24, X 2.

similar to *H. escragnollense* (Breistroffer, 1947, p 71 = *Phylloceras ellipticum* Parona and Bonarelli, 1897, Pl. 1, Figs. 7abc, *non* Kossmat, 1895). All that can be said about the St. Thomas specimen, then, is that it belongs to that Albian group of *Hypophylloceras* spp., which is characterized by a more rapidly expanding whorl that does not taper ventrad as does the whorl of *H. subalpinus* (d'Orbigny), but is fully rounded as in *H. ellipticum* (Kossmat) and *H. escragnolles* (Breistroffer). In 1972 I incorrectly compared this specimen to *Hypophylloceras* sp. cf. *H. velledae* (Michelin, 1838).

*Horizon and Locality.* The specimen of *Hypophylloceras* from St. Thomas, Virgin Islands, is from the Tutu Formation, west end of the Island. The age is Albian, as already pointed out by Donnelly (1970b), but can be determined as Upper Albian on the basis of 2 associated specimens of *Mortoniceras*.

Suborder Lytoceratina Hyatt, 1889
Superfamily Lytoceratacea Neumayr, 1875
Family Tetragonitidae Hyatt, 1900
Subfamily Gaudryceratinae Spath, 1927
Genus *Eogaudryceras* Spath, 1927
*Eogaudryceras* (?) sp.

Pl. 1, Figs. 4, 5; Pl. 2, Figs. 1-8

*Dimensions.*

| Specimen | D | H | W | U | H/W |
|---|---|---|---|---|---|
| WSA-16301 | 48.0 | 54.5 | 58.5 | 24.0 | 0.93 |
| | 33.5 | 49.0 | 51.0 | 18.7 | 0.96 |
| | 23.5 | 49.0 | 62.0 | 27.5 | 0.79 |

*Remarks.* According to Wiedmann (1962b) the primary difference between *Eogaudryceras* and *Tetragonites* is the suture, not recoverable on the Puerto Rican specimens, and according to Murphy (1967) the extremely prosiradiate restrictions in *Tetragonites*. There are no restrictions on the Puerto Rican specimens, which would indicate their assignment to *Eogaudryceras*, except that many specimens of *Tetragonites*, much better preserved than those from Puerto Rico, do not show the constrictions (Wiedmann, 1962b, Pl. 14). Perhaps their absence is due to poor preservation of the fossils.

That *Tetragonites* has a more symmetrical first lateral lobe and a broader and better developed suspensive lobe than *Eogaudryceras* seems undoubted (Wiedmann, 1962b). Furthermore, *Eogaudryceras* lacks a good internal lateral lobe, whereas *Tetragonites* has the well developed internal lateral lobe. But these features cannot be recovered on the material from Puerto Rico.

Some lineages of *Tetragonites* become more depressed through the Albian [e.g., lineage of *T. timotheanus* (Pictet, 1848)], whereas *Eogaudryceras*, leading to *Gaudryceras*, becomes less depressed. The Puerto Rican specimens are less

depressed than *T. timotheanus*, but more depressed than most specimens of *Eogaudryceras*, and some of them have slightly trapezoidal whorls sections, leading one to believe that they are not far from the divercation of the 2 genera, and thus early in the generic lineages (e.g., probably Lower Albian or very earliest Middle Albian).

*Horizon and Locality.* All specimens are from the same locality and horizon, pre-Robles beds, about 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087 (= U.S.G.S. locality 26804), from a road cut on secondary road 0.85 mi ESE of Escuela Segunda Unidad de la Lama and about 2.9 mi airline due north of Aiboneto, Borrio Hunduras, Municipio de Barranquitas, Puerto Rico meter grid 170, 175 X 38, 700, Barranquitas Quadrangle (Sohl, 1975).

Suborder Ammonitina
Superfamily Desmoceratacеae Zittel, 1895
Family Desmoceratidae Zittel, 1895
Subfamily Puzosiinae Spath, 1922
Genus *Puzosia* Bayle, 1878

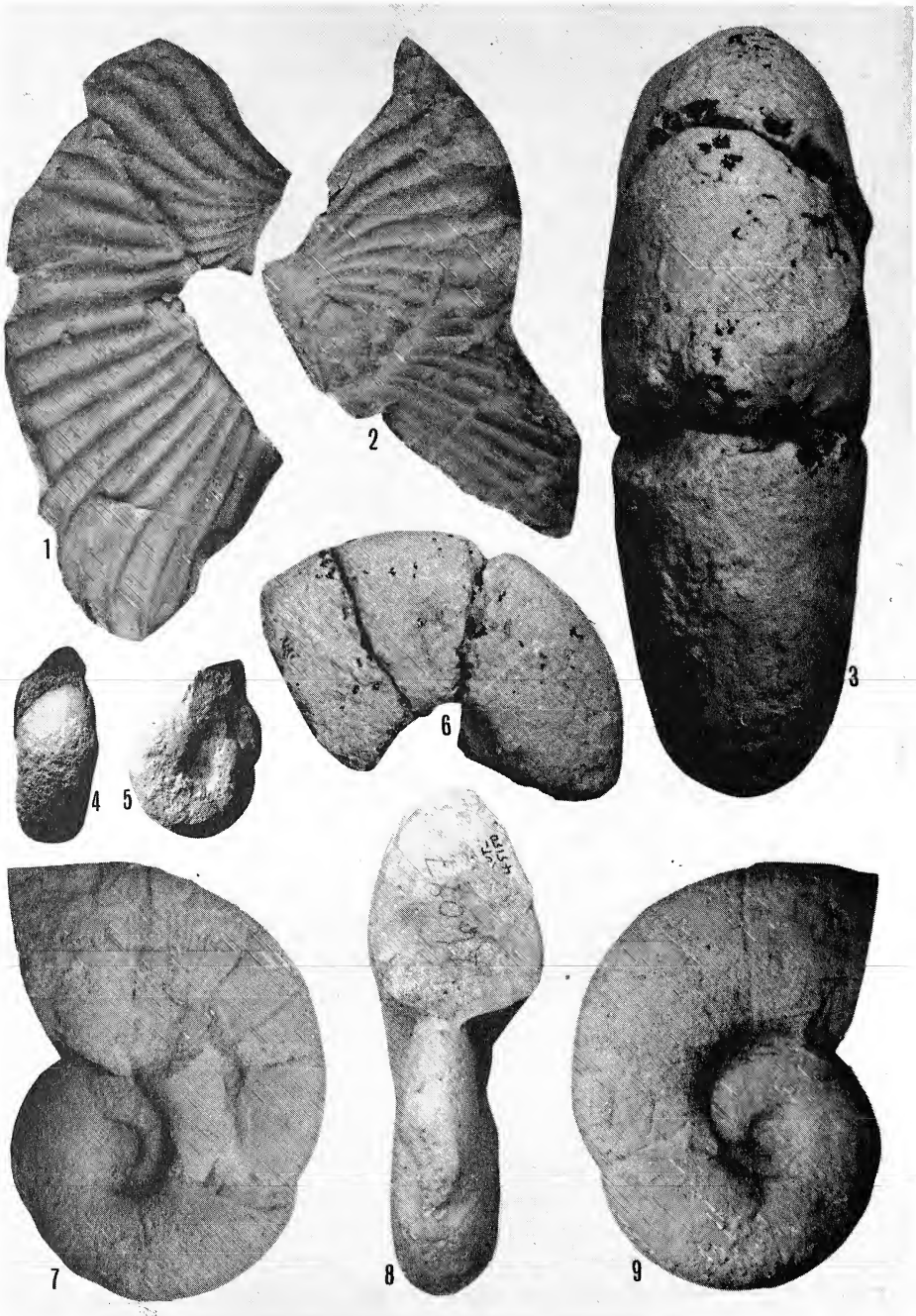
*Puzosia* sp. cf. *P. welwitschi* Choffat and de Loriol, 1888.
Pl. 1, Figs. 3, 6-9; Pl. 2, Figs. 13, 16-18
=*Puzosia* sp. cfr. *planulata* Bayle, 1878 in Young, 1972, p. 469
*cf. Puzosia welwitschi* Choffat and de Loriol, 1888, Pl. 2, Fig. 3.
*cf. Desmoceras cuvervillei* Meunier, 1888, Pl. 1, Fig. 3.

*Dimensions.*

| Specimen | D* | H | W | U | H/W |
|---|---|---|---|---|---|
| WSA-16302 | 160.0 | 42.0 | 33.7 | 24.5 | 1.25 |
| | 100.0 | 45.0 | 35.5 | 24.5 | 1.27 |
| | 60.0 | 48.5 | 36.5 | 24.5 | 1.33 |
| WSA-16304 | | 49.5* | 39.0* | | 1.27 |
| WSA-16306 | 76.5 | 49.0 | 36.0 | 28.0 | 1.36 |
| | 45.5 | 48.5 | 37.5 | 25.0 | 1.29 |

*Figures marked with an asterisk are in mm. Other figures are percentages or a ratio (H/W).

*Remarks.* Matsumoto's (1954) remarks on the differences between *Puzosia (Anapuzosia)* and *Puzosia (Puzosia)* give one the impression that the two subgenera can only be differentiated on large specimens. If the statement concerning the age of the Puerto Rican specimens of *Eogaudryceras* (?) sp. is correct, then the Puerto Rican fauna from Aiboneto is too old to include *Puzosia planulata* Bayle, or most other specimens of *Puzosia*. Matsumoto (1954) does point out that *P. planulata* has, at least in some younger stages, a higher whorl section than do specimens that can definitely be referred to *Anapuzosia* (e.g., *P. mayor-*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## DESCRIPTION OF PLATE 1

1, 2 *Manuaniceras* sp. cf. *M. supani* (Lasswitz), lateral views of latex peels of 2 uncollectable specimens (WSA 16297) from the Robles Formation, near the middle of the bedded sequence on the Convento Road north of Comerio, Comerio Quadrangle, Puerto Rico.

3, 6–9 *Puzosia* sp. cf. *P. welwitschi* Choffat and de Loriol, 1888, 3, 6, lateral and ventral views of WSA 16304; 7–9, lateral and ventral views of WSA 16302 (see also Pl. 2, Fig. 17); both from pre-Robles formation beds about 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087.

4, 5 *Eogaudryceras* ? sp. lateral and ventral views of WSA 16308, from the pre-Robles beds about 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087.
1–5, X 1.0; 6, X 0.5; 7–9, X 0.4.

*iana* Jacob, 1908, Pl. 16, Fig. 2, not d'Orbigny). The Puerto Rican specimens are all higher than wide, H/W ranging from 1.25 to 1.35, although the specimens with higher ratios may be flattened by sedimentary load. Constrictions are more frequent than in *Puzosia (Puzosia) mayoriana* (d'Orbigny) (Spath, 1923, Text-fig. 10, Pl. 1, Figs. 9ab, 10ab). The Puerto Rican specimens have an evenly arched venter, compressed whorl section, and vertical umbilical wall. Furthermore, the constriction is a definite sulcus following a major rib that truncates normal ribbing dorsad. The constrictions do not have the linguiform ventrad projection of *P. mayoriana* (Spath, 1923, pp 43, 44, Text-Fig. 10), *P. planulata* Bayle, or *P. crebrisulcata* Kossmat (1898, Pl. 17, Figs. 4ab), but are more like those of *P. welwitschi* Choffat and de Loriol (1888, Pl. 2, Fig. 3), but less sigmoid. They are nearly straight up to a diameter of 80 mm., more like those of *P. cuvervillei* (Meunier, 1888, Pl. 1, Fig. 3), but the latter specimen is so small as to prevent proper identification.

*Horizon and Locality.* All specimens are from the same locality and horizon, pre-Robles beds, about 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087 [see more complete description under *Eogaudryceras* (?) sp.].

The species with which the Aiboneto specimens are compared are mostly Upper Albian, and *Puzosia* does not range below the Middle Albian. Choffat and de Loriol (1888, p. 23) list *P. welwitschi* as occurring in the same beds with *Pervinquieria inflata* and *Douvilleiceras* [*Acanthoceras mamillare* (Schloth.)? of Choffat and de Loriol, 1888, p 24, Pl. 3, Figs. labc], without indication of relative age. It could be as low as Middle Albian, but they list no Lower Albian fossils. Meunier (1888, p 62) likewise lists *P. cuvervillei* with "*Schloenbachia inflata*, Sow", but his "*S. inflata*" (Pl. 1, Fig. 1, not Fig. 2) appears to be a *Mosjisovicsia*, and is probably Middle Albian. Since there is no evidence of large ribs or horns in the adult stages of the Puerto Rican form, it does not seem advisable to assign it to *Anapuzosia*. The 2 species to which it can best be compared both seem to be Middle Albian, although an Upper Albian age for *P. welwitschi* Choffat and de Loriol is not eliminated.

Superfamily Hoplitaceae H. Douville, 1890
Family Engonoceratidae Hyatt, 1900
Genus *Engonoceras* Neumayr & Uhlig, 1881
*Engonoceras* (?) spp.
Pl. 2, Figs, 9-12, 14, 15

*Remarks.* There are at least 2 species of *Engonoceras* in the collection from near Aiboneto, P.R., a species with an acute keel (Pl. 2, Figs. 9-12) and a tabulate species (Pl. 2, Figs. 14, 15). The smaller specimen of the species with an acute keel shows very faint, ventrad costae, as in the juveniles of *Engonoceras stolleyi* Bohm (Hyatt, 1903, Pl. 24, Fig. 4), but unlike *E. stolleyi* this specimen would appear to have an acute keel. Many engonocerids have tabulate venters, and the fragment illustrated herein (Pl. 2, Figs. 14, 15) is not further identifiable.

*Horizon and Locality.* All specimens are from the same locality and horizon, pre-Robles beds, about 3 mi north of Aiboneto, Puerto Rico, U.S.G.S. Mesozoic locality 26087 [see more complete description under *Eogaudryceras* (?) sp.].

Superfamily Acanthocerataceae Hyatt, 1900
Family Brancoceratidae Spath, 1933
Genus *Manuaniceras* Spath, 1925a

*Manuaniceras* sp. cf. *M. supani* (Lasswitz)
Pl. 1, Figs. 1, 2

=*Manuaniceras* sp. cf. *supani* (Lasswitz) in Young, 1972, p 469.
For synonomy of *M. supani* (Lasswitz) see Young, (1966), p 103.

*Remarks. Manuaniceras supani* (Lasswitz) is differentiated from other species of *Manuaniceras* by its ribs overhanging the adjacent sulcus during some stage of growth. Otherwise, the shells are moderately evolute with high whorls (H/W ranging from 1.5 in juveniles to 2.4 or more in larger specimens), and they have ribs that range from sigmoid to straight, with a forward swing near the venter; ribs do not join the keel.

The pictures of the Puerto Rican specimens are lateral views only, but show at some growth stages the walls of the ribs overhanging the sulcus, a feature diagnostic of the species.

In older members of the species the overhang is restricted to a rather narrow growth stage, but in younger members rib overhang may occur throughout that ontogeny beyond a diameter of 60 mm. The Puerto Rican form belongs to the older part of the lineage and is from high in the Middle Albian, according to zonation schemes of Breistroffer (1947) or Young (1966), or older, but not Lower Albian.

*Horizon and Locality.* From the Robles Formation near the middle of the bedded sequence on the Convento Road north of Comerio, Comerio Quadrangle, Puerto Rico.

The indicated age is Middle Albian and most likely the upper part of the Middle Albian.

Subfamily Mortoniceratinae Spath, 1925
Genus *Mortoniceras* Meek, 1876

*Mortoniceras* sp. juv. sp. cf. *M. nanum* Spath, 1933
Pl. 2, Figs. 21, 22

=*Mortoniceras* sp. juv. cf. *M. minor* Spath in Young, 1972, p 469.
*cf. Mortoniceras (Pervinquieria?) nanum* Spath, 1933, Pl. 43, Figs. 6ab.

*Dimensions.*

| H | W | H/W |
|---|---|---|
| 4.2* | 5.4* | 0.78 |

*These figures are in mm.

*Remarks.* The specimens of *Mortoniceras* from St. Thomas are too small to identify specifically. Although not readily visible in the figure, this specimen has binodose ribs bifurcating rectiradially from the umbilical node--a feature very typical of *Mortoniceras.*

*Horizon and Locality.* From the Tutu Formation, west end of the island of St. Thomas, Virgin Islands. The specimen is in the possession of T.W. Donnelly.

*Mortoniceras* sp. juv. sp. cf. *M. scobinum*
(Van Hoepen, 1942)
Pl. 2, Figs. 19, 20

=*Mortoniceras* sp. juv. cf. *scotia* (typographical error) (Van Hoepen) in Young, 1972, p 469.

cf. *Pervinquieria scobina* Van Hoepen, 1942, p 111, Figs. 92 and 93.

*Dimensions.*

| H | W | H/W |
|---|---|---|
| 5.1* | 6.1* | 0.83 |

*These figures are in mm.

*Remarks.* This specimen is more densely costate than the specimen compared to *M. nanum* Spath; its ribs are slightly sigmoid, and swing more ventrad near the venter. It likewise is too juvenile to identify accurately, but is typical of minute specimens of *Mortoniceras* that can be found in western Europe, Angola, Madagascar, and Texas. They are always Upper Albian. One reason that identification is difficult is because such small binodose juveniles may become trinodose prior to a diameter of 50 mm--*Pervinquieria* and some *Deiradoceras*--or they may remain binodose to diameters of 200 mm or more--*Mortoniceras* (=*Leonites* Spath, 1932).

*Horizon and Locality.* From the Tutu Formation, west end of the island of St. Thomas, Virgin Islands. The specimen is in the possession of T.W. Donnelly.

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# IDEAL GAS THERMODYNAMIC FUNCTIONS OF THE 2-HALOPROPENES

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ABSTRACT

The thermodynamic functions Gibbs free energy function, enthalpy function, entropy, and heat capacity were calculated for 2-fluoropropene, 2-chloropropene, 2-bromopropene, and 2-iodopropene in the ideal gas state at one atm pressure and selected temperatures.

## INTRODUCTION

Thermodynamic properties of the 2-halopropenes have not been reported in the literature. Vapor-state infrared spectra and barriers to internal rotation of the methyl groups have recently become available for the 2-halopropenes, and these data have made possible the calculation of ideal gas thermodynamic functions for these compounds. The functions $(G^\circ - H^\circ_0)/T$, $(H^\circ - H^\circ_0)/T$, $S^\circ$, and $C^\circ_p$ were calculated for each of the four 2-halopropenes in the ideal gas state at 1 atm pressure and selected temperatures. The contributions of translation, over-all rotation, and vibration were calculated with standard formulas of statistical thermodynamics (Pitzer, 1953). These calculations were based on the rigid rotator-harmonic oscillator model. The equations that were used are summarized in the Appendix. The contributions of restricted internal rotation of the methyl groups were taken from the tables of Pitzer and Gwinn (1942).[1] No corrections were made for anharmonicity, etc., because no experimental heat capacity or entropy data were available for use in making those corrections.

## RESULTS

### *2-Fluoropropene*

Pierce and O'Reilly (1959) have obtained moments of inertia of this molecule from the microwave spectrum, and have calculated a methyl torsional barrier of 2432 cal/mole. However, they used the value 3.11 amu Å$^2$ for the moment of

[1] A computer program that obtains the contributions of restricted internal rotation from the Pitzer and Gwinn tables has been described by Crowder and Riley (1972).

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inertia, $I_\alpha$, of the methyl group about its axis, a value they assumed to be the same as in propene. However, Fateley and Miller (1963) pointed out that an accurate value of the structural factor F can be obtained from the torsional frequency and the value of the reduced barrier height, s, obtained from microwave work. Fateley and Miller's (1963) value of F = 5.620 $cm^{-1}$ yielded $I_\alpha$ = 3.205 amu Å$^2$ and a methyl barrier ($V_3$) of 2340 cal/mole. The reduced moment of inertia[2] of the methyl group was calculated from their $I_\alpha$ to be 3.00 amu Å$^2$. (An uncertainty of 0.05 amu Å$^2$ in the reduced moment results in an uncertainty of 0.04 cal or less in the thermodynamic functions.) The vibrational assignment was taken from Crowder and Smyrl (1971). The molecular data used in the calculations are listed in Table 1 and the thermodynamic functions for 2-fluoropropene at selected temperatures are given in Table 2.

TABLE 1
Molecular Constants of 2-Fluoropropene

| Property | Value |
|---|---|
| Molecular weight | 60.071 |
| Principal moments of inertia | |
| $I_A$ (amu Å$^2$) | 49.71 |
| $I_B$ (amu Å$^2$) | 55.91 |
| $I_C$ (amu Å$^2$) | 102.60 |
| Reduced moment of inertia (amu Å$^2$) | 3.00 |
| Potential barrier, $V_3$ (cal/mole) | 2340 |
| Vibrational frequencies ($cm^{-1}$) | 3141, 3059, 3012<br>2975, 2942, 1687<br>1448, 1448, 1429<br>1401, 1270, 1048<br>1008, 944, 862<br>846, 629, 472<br>404, 352 |

*2-Chloropropene*

Unland, *et al.*, (1965) have determined the principal moments of inertia of $CH_2 = C^{35}ClCH_3$ and $CH_2 = C^{37}ClCH_3$. These authors gave a value of 3.110 amu Å$^2$ for $I_\alpha$, which is the result of their assumed value of 111.20° for the C-C-H angle involving the methyl hydrogens. From their F value (5.62 $cm^{-1}$), we calculated a reduced moment of inertia of the methyl group of 3.00 amu Å$^2$. The barrier height obtained by Unland, *et al.*, (1965) was used in the calculations,

[2]The relation between the reduced moment of inertia, $I_r$, and $I_\alpha$ is

$$I_r = I_\alpha[1 - I_\alpha \sum_g (\lambda_g / I_g)]$$

where $\lambda_g$ is the cosine of the angle between the axis of the internal top and the $g^{th}$ principal axis and $I_g$ is the $g^{th}$ principal moment of inertia.

TABLE 2

Thermodynamic Functions for 2-Fluoropropene in the Ideal Gas State at 1 atm

| T | $-(G^o-H_O^o)/T$ | $(H^o-H_O^o)/T$ | $S^o$ | $C_p^o$ |
|---|---|---|---|---|
| K | cal/deg/mole | cal/deg/mole | cal/deg/mole | cal/deg/mole |
| 273.15 | 55.53 | 11.64 | 67.17 | 17.19 |
| 298.15 | 56.59 | 12.14 | 68.73 | 18.25 |
| 300 | 56.66 | 12.18 | 68.84 | 18.33 |
| 400 | 60.44 | 14.22 | 74.66 | 22.25 |
| 500 | 63.83 | 16.17 | 80.00 | 25.64 |
| 600 | 66.94 | 18.00 | 84.94 | 28.50 |
| 700 | 69.85 | 19.67 | 89.52 | 30.91 |
| 800 | 72.56 | 21.22 | 93.78 | 32.99 |
| 900 | 75.15 | 22.62 | 97.77 | 34.78 |
| 1000 | 77.61 | 23.91 | 101.52 | 36.32 |

and the vibrational assignment was taken from Hunziker and Gunthard (1965). These latter authors used a value of F = 5.502 $cm^{-1}$ to calculate the barrier height, but they calculated this value from structural data transferred from propylene and vinyl chloride. The F value obtained by Unland, *et al.*, (1965) is considered more accurate since it is the same as for 2-fluoropropene and very nearly the same as for 2-bromopropene (next section).

The molecular constants used for 2-chloropropene are listed in Table 3 and the thermodynamic functions are given in Table 4.

TABLE 3

Molecular Constants of 2-chloropropene

| Property | Value | |
|---|---|---|
| Molecular weight | 76.526 | |
| Principal moments of inertia | | |
| $I_A$ (amu Å$^2$) | 54.52[a] | 54.52[b] |
| $I_B$ (amu Å$^2$) | 101.43[a] | 104.22[b] |
| $I_C$ (amu Å$^2$) | 152.99[a] | 155.78[b] |
| Reduced moment of inertia (amu Å$^2$) | 3.00 | |
| Potential barrier, $V_3$ (cal/mole) | 2671 | |
| Vibrational frequencies ($cm^{-1}$) | 3121, 3025, 2992, 2973<br>2940, 1645, 1450, 1450<br>1424, 1382, 1184, 1046<br>999, 926, 879, 692<br>641, 434, 396, 343 | |

[a]Moments of inertia for $CH_2 = CCl^{35}CH_3$

[b]Moments of inertia for $CH_2 = CCl^{37}CH_3$

TABLE 4

Thermodynamic Functions for 2-Chloropropene in the Ideal Gas State at 1 atm

| T °K | $-(G^\circ-H_0^\circ)/T$ cal/deg/mole | $(H^\circ-H_0^\circ)/T$ cal/deg/mole | $S^\circ$ cal/deg/mole | $C_p^\circ$ cal/deg/mole |
|---|---|---|---|---|
| 273.15 | 57.36 | 11.76 | 69.12 | 17.62 |
| 298.15 | 58.42 | 12.29 | 70.71 | 18.72 |
| 300 | 58.48 | 12.34 | 70.82 | 18.78 |
| 400 | 62.33 | 14.46 | 76.79 | 22.72 |
| 500 | 65.79 | 16.47 | 82.26 | 26.17 |
| 600 | 68.94 | 18.33 | 87.27 | 28.98 |
| 700 | 71.91 | 20.02 | 91.93 | 31.33 |
| 800 | 74.68 | 21.57 | 96.25 | 33.36 |
| 900 | 77.31 | 22.98 | 100.29 | 35.10 |
| 1000 | 79.79 | 24.26 | 104.05 | 36.62 |

*2-Bromopropene*

Benz, *et al.*, (1966) determined, by microwave spectroscopy, the principal moments of inertia of $CH_2 = C^{79}BrCH_3$ and $CH_2 = C^{81}BrCH_3$. The reduced moment of inertia was calculated from their F value of 5.60 $cm^{-1}$, and their barrier height was used for the methyl torsion.The barrier has also been calculated from the infrared torsional frequency (Crowder and Waltrip, 1977), and the 2 values agree within the uncertainties involved.

A vibrational assignment has been made for 2-bromopropene by Meyer and Gunthard (1967). The far infrared spectrum has also been obtained by one of the present authors in the region below 650 $cm^{-1}$ with a Perkin-Elmer 301 far infrared spectrophotometer, through the courtesy of Dr. Donald W. Scott. Our frequencies agree well with those of Meyer and Gunthard, except for the C=C-C bending vibration. This is a type B band with branches at 343 and 355 $cm^{-1}$ and a maximum between the 2 branches, at 350 $cm^{-1}$ in the vapor-state spectrum. Our liquid-state value is 351 $cm^{-1}$, and the liquid-state Raman value is 354 $cm^{-1}$ (Kirrman, 1939). The value reported by Meyer and Gunthard, (1967) is 335 $cm^{-1}$, which differs significantly from our value. The spectrum shown by these authors shows this band at ca. 350 $cm^{-1}$, so the 335 $cm^{-1}$ value they reported is probably a typographical error. The other frequencies below 650 $cm^{-1}$ differ by 2 $cm^{-1}$ at the most.

The molecular constants used in the calculations are listed in Table 5. The vibrational frequencies above 600 $cm^{-1}$ were taken from Meyer and Gunthard (1967) and those below 600 $cm^{-1}$ were taken from this work. The resulting thermodynamic functions are given in Table 6.

*2-Iodopropene*

Structural data have not been obtained for 2-iodopropene, so the molecular parameters were transferred from 2-chloropropene (Unland, *et al.*, 1965), and

TABLE 5
Molecular Constants of 2-Bromopropene

| Property | Value | |
|---|---|---|
| Molecular weight | 120.98 | |
| Principal moments of inertia | | |
| $I_A$ (amu Å$^2$) | 54.62[a] | 54.62[b] |
| $I_B$ (amu Å$^2$) | 161.10[a] | 162.40[b] |
| $I_C$ (amu Å$^2$) | 212.78[a] | 214.08[b] |
| Reduced moment of inertia (amu Å$^2$) | 3.01 | |
| Potential barrier, $V_3$ (cal/mole) | 2695 | |
| Vibrational frequencies (cm$^{-1}$) | 3115, 3010, 2987<br>2972, 2930, 1640<br>1443, 1439, 1405<br>1379, 1170, 1045<br>996, 925, 883, 680<br>550, 412, 350, 299 | |

[a]Moments of inertia for $CH_2 = CBr^{79}CH_3$
[b]Moments of inertia for $CH_2 = CBr^{81}CH_3$

TABLE 6
Thermodynamic Functions for 2-Bromopropene in the Ideal Gas State at 1 atm

| $\frac{T}{^oK}$ | $\frac{-(G^o-H^o_O)/T}{cal/deg/mole}$ | $\frac{(H^o-H^o_O)/T}{cal/deg/mole}$ | $\frac{S^o}{cal/deg/mole}$ | $\frac{C^o_p}{cal/deg/mole}$ |
|---|---|---|---|---|
| 273.15 | 59.75 | 12.13 | 71.88 | 18.12 |
| 298.15 | 60.84 | 12.67 | 73.51 | 19.19 |
| 300 | 60.91 | 12.71 | 73.62 | 19.26 |
| 400 | 64.86 | 14.85 | 79.71 | 23.17 |
| 500 | 68.40 | 16.85 | 85.25 | 26.47 |
| 600 | 71.64 | 18.69 | 90.33 | 29.22 |
| 700 | 74.64 | 20.37 | 95.01 | 31.54 |
| 800 | 77.47 | 21.88 | 99.35 | 33.52 |
| 900 | 80.12 | 23.28 | 103.40 | 35.25 |
| 1000 | 82.64 | 24.55 | 107.19 | 36.74 |

the C-I bond length was assumed to be the same as in vinyl iodide, 2.089 Å (Chemical Society, 1958). The principal moments of inertia were calculated from those structural parameters. For internal rotation of the methyl group, the F value was taken to be 5.60 cm$^{-1}$, which is the value for 2-bromopropene. The F value for 2-fluoropropene and 2-chloropropene, 5.62 cm$^{-1}$, is only slightly higher. The barrier height has been calculated by Crowder and Waltrip (1977)

from the observed torsional frequency. The vibrational frequencies were taken from Meyer, *et al.*, (1969). The molecular constants are listed in Table 7 and the thermodynamic functions are given in Table 8.

TABLE 7
Molecular constants of 2-Iodopropene

| Property | Value |
|---|---|
| Molecular weight | 167.98 |
| Principal moments of inertia | |
| $I_A$(amu Å$^2$) | 53.23 |
| $I_B$(amu Å$^2$) | 218.83 |
| $I_C$(amu Å$^2$) | 268.87 |
| Reduced moment of inertia (amu Å$^2$) | 3.01 |
| Potential barrier, $V_3$ (cal/mole) | 2580 |
| Vibrational frequencies (cm$^{-1}$) | 3103, 2994, 2974<br>2967, 2928, 1627<br>1457, 1436, 1406<br>1377, 1159, 1056<br>991, 926, 893, 701<br>514, 389, 318, 271 |

TABLE 8
Thermodynamic Functions for 2-Iodopropene in the Ideal Gas State at 1 atm

| T<br>°K | $-(G^o-H^o_0)/T$<br>cal/deg/mole | $(H^o-H^o_0)/T$<br>cal/deg/mole | $S^o$<br>cal/deg/mole | $C^o_p$<br>cal/deg/mole |
|---|---|---|---|---|
| 273.15 | 61.47 | 12.38 | 73.85 | 18.35 |
| 298.15 | 62.58 | 12.92 | 75.50 | 19.37 |
| 300 | 62.67 | 12.96 | 75,63 | 19.44 |
| 400 | 66.68 | 15.07 | 81.75 | 23.25 |
| 500 | 70.26 | 17.04 | 87.30 | 26.50 |
| 600 | 73.54 | 18.84 | 92.38 | 29.24 |
| 700 | 76.56 | 20.50 | 97.06 | 31.55 |
| 800 | 79.39 | 22.01 | 101.40 | 33.54 |
| 900 | 82.07 | 23.39 | 105.46 | 35.26 |
| 1000 | 84.61 | 24.65 | 109.26 | 36.75 |

## CONCLUSION

Ideal gas thermodynamic functions have been calculated for the 2-halopropenes at 1 atm pressure. There are no experimental data available for comparisons, but calculations have yielded accurate values for other compounds. Recently, for

example, Chen, *et al.*, (1975) used this method to calculate the thermodynamic functions for $CF_3CH_3$, which has only 1 atom fewer than the 2-halopropenes. Their calculated entropy of 63.82 cal $K^{-1}$ $mol^{-1}$ at 224.4K agrees well with the experimental value of 63.89 cal $K^{-1}$ $mol^{-1}$ determined by Russell, *et al.*, (1944). The calculated entropy of $CF_3CF_3$ at 2 temperatures (Chen, *et al.*, 1975) also agrees well with the experimental values of Pace and Aston (1948).

Thermodynamic functions were calculated for both isotopic species of 2-chloro and 2-bromopropene and were averaged according the isotopic abundance. The difference between the functions of the 2 species amounted to only 0.04 cal for 2-chloropropene and 0.01 cal for 2-bromopropene.

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APPENDIX

The equations used to calculate the thermodynamic functions are summarized here. All equations result in calorie units.

*Heat Capacity*

translation, rotation, and vibration:

$$C_p^\circ = 7.9487 + \sum_{i=1}^{20} 1.4388\,(\bar{\nu}_i/T)e^{1.4388\bar{\nu}_i/T}/(e^{1.4388\bar{\nu}_i/T}-1)^2$$

*Enthalpy function*

translation, rotation, and vibration:

$$\frac{H^\circ - H_0^\circ}{T} = 7.9487 + \sum_{i=1}^{20} 1.9872T/(1-e^{-1.4388\bar{\nu}_i/T})$$

*Entropy*

translation, rotation, free internal rotation, and vibration:

$$S^\circ = (18.3025 + 2.2878n)\log T - 2.3493 + 3.0346n + 6.8634 \log M$$

$$+ 2.2878 \log \{I_1 I_2 I_3 \times 10^{117}[\prod_{r=1}^{n} (I_r \times 10^{38})]\} - 4.5756 \log (\sigma \prod_{r=1}^{n} \sigma_r)$$

$$+ \sum_{i=1}^{20} \frac{2.8591\bar{\nu}_i/T}{e^{1.4388\bar{\nu}_i/T}} - 4.5756 \log (1-e^{-1.4388\bar{\nu}_i/T})$$

*Gibbs Free Energy Function*

The Gibbs free energy function was obtained from the entropy and enthalpy function:

$$-\left(\frac{G^\circ - H_o^\circ}{T}\right) = S^\circ - \left(\frac{H^\circ - H_o^\circ}{T}\right)$$

*Restricted Internal Rotation*

The restricted internal rotational contributions were taken from the tables of Pitzer and Gwinn (1942), which have as variables $V/RT$ and $1/Q_f$, where V is the barrier to internal rotation and $Q_f$ is the partition function for free internal rotation. The tables for heat capacity and enthalpy function lists the direct contribution of the internal rotation, but the table for entropy lists the decrease from free internal rotation.

*Definitions of Variables in the Equations*

n = number of internal rotations

T = absolute temperature

$\bar{\nu}_i$ = the $i^{th}$ vibrational wavenumber ($cm^{-1}$)

M = molecular weight (g)

$I_1 I_2 I_3$ = product of the three principal moments of inertia ($g\ cm^2$)

$I_r$ = reduced moment of inertia of the $r^{th}$ rotating group ($g\ cm^2$)

$\sigma$ = symmetry number for overall rotation

$\sigma_r$ = symmetry number for the $r^{th}$ internal rotation

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# A PROTOSTEGID (SEA TURTLE) FROM THE TAYLOR FORMATION OF TEXAS

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ABSTRACT

A right hypoplastron recovered from the Pecan Gap Chalk of the Taylor Formation in Collin County, Texas is the fourth reported chelonioid in the Cretaceous of Texas. This specimen is referred to the family Protostegidae but generic assignment is not made. The other Texas specimens are from the Eagle Ford Formation (Zangerl, 1953), the Austin Chalk (McNulty and Slaughter, 1964) and the Taylor Formation (Zangerl, 1953).

## INTRODUCTION

In 1974, the author received a well preserved portion of a right hypoplastron which was recovered from the Taylor Formation (Gulfian) of Collin County, Texas. The locality is an outcrop 500 m east of Lake Lavon Dam where the Pecan Gap Chalk is exposed. It is on deposit in the collections of the Shuler Museum of Paleontology at Southern Methodist University (SMP-SMU 63472).

## DESCRIPTION

The specimen consists of a major portion of the right hypoplastron and includes a wedge of the right xiphiplastron (Fig. 1). The dorsal concavity is preserved with a relief of 4.3 mm and is nearly symmetrical about a central point on the hypoplastron. From this point, narrow elongate ridges radiate out to the extremeties of the bony plate. These ridges can be traced on the ventral surface as well. The insertion of the xiphiplastron into the hypoplastron produces a ridge on the dorsal and ventral surfaces. This ridge originates along the suture where a portion of the xiphiplastral element is exposed on the dorsal surface. This insertion, as traced by the ridge, cannot be traced to the central point but extends about half way (Table 1). This ridge is delineated in Figure 1 by narrow, elongate depressions.

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Figure 1. Dorsal view of Protostegidae right hypoplastron with dorsal concavity exposed; arrow points anterior.

TABLE 1
Measurements

| | |
|---|---|
| Anterior–Posterior Diameter | 37.6 mm |
| Lateral Diameter | 30.7 mm |
| Maximum Thickness | 1.86 mm |
| Minimum Thickness | 0.52 mm |
| Length of Xiphiplastoral Insertion Ridge | 9.0 mm |

The hypoplastron is not complete as preserved. The internal edge which extends into the central cavity of the plastron is relatively thin, indicating a termination and demonstrating that this is a natural edge (Fig. 2b). The external edge is damaged and the greatest thickness is measured along this fracture. The natural and fractured edges are indicated in Figure 2a.

## COMPARISON

The properties of the Superfamily Chelonioidea are self evident. A comparison of the right hypoplastron with a representative of each of the 3 families

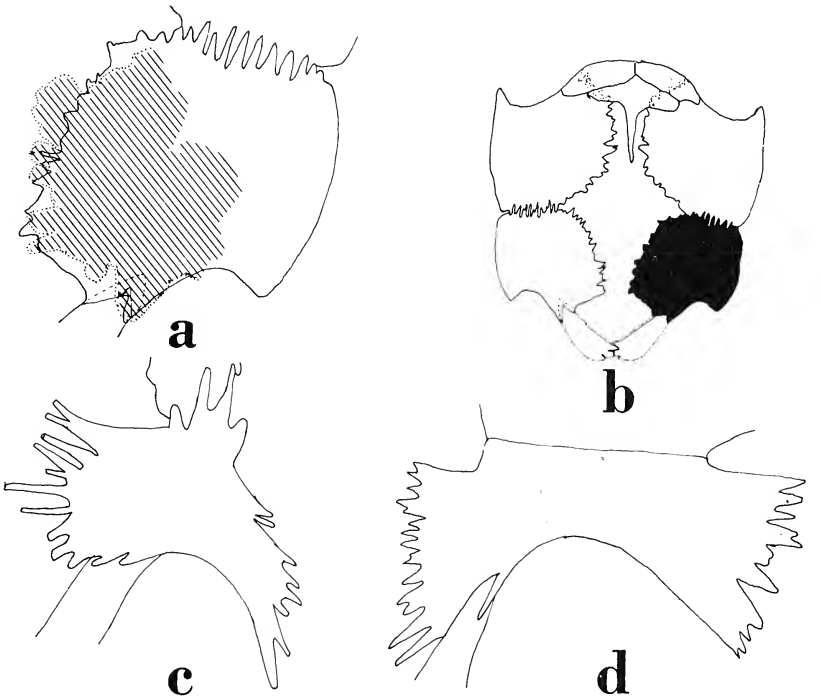


Figure 2. Dorsal view of upper Cretaceous Chelonioidea plastral plates. (Modified from Zangerl, 1953) a. Protostegidae showing the alignment of our fossil specimen (shaded); dotted line indicates natural edge. Chelosphargis advena. b. Outline of Protostegidae plastron; right hypoplastron darkened. e. Cheloniidae right hypoplastron. Corsoehelys haliniches. d. Toxochelyidae right hypoplastron. Toxochelys latiremis.

suggests a protostegid affinity. This relationship was established using the overall geometry and the character of the insertion of the xiphiplastron. The right hypoplastron of the Protostegidae, Toxoehelyidae, and Cheloniidae are reproduced from Zangerl (1953) (Fig. 2). A photograph of our specimen was aligned over these drawings using the xiphiplastral insertion as a guide.

The toxochelyid xiphiplastral insertion is developed in a proximal position to the internal edge (Fig. 2d). The cheloniid xiphiplastron exhibits a relatively wider insertion and the anterior-posterior length is not as well developed as in our fossil specimen (Fig. 2c).

The fossil right hypoplastron is assigned to the Protostegidae of which three genera are described (Zangerl, 1953). Generic assignment of this specimen must wait for additional Gulfian protostegids.

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# CHOICE OF MINICOMPUTERS' FLOATING POINT BASE

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ABSTRACT

The large increase in usage of the minicomputer for scientific applications has generated a new flurry of discussion of the relative numerical merits of various choices of the base for floating point arithmetic systems. Most of the papers on this subject compare usage of binary, quarternary, and hexadecimal formats with the range of the exponent remaining a common factor. This then leads to comparison of 32 bit (Fraction Bits X Exponent Bits) floating point formats of (23 X 9), 24 X 8), and (25 X 7) for binary, quarternary, and hexadecimal bases respectively. This paper compares the numerical precision attainable using various bases with a common word structure for all bases. In addition, comparison of accuracies for common existing floating point systems are given.

## INTRODUCTION

Over the past few years, the minicomputer has progressed from being a hardware device used primarily for monitor and control, to a widely accepted computer for general applications. This change has been primarily generated either because of its low cost or because of small physical size requirements as is found in many military applications. In any case, scientific programmers are often finding the minicomputer with its small word size their primary tool for problem solving. Thus, discussion of the numerical accuracy of these computers has been active. In particular, several recent papers, e.g. Brent (1973) and Cody (1973), discuss the relative merits of various floating point formats and choices of base for a 32 bit floating point word.

Several floating point bases are currently being used, including binary, octal, and hexadecimal. In addition, a base 4, or quarternary number representation scheme has been proposed. Comparisons of numerical accuracy as a function of the base used have, for the most part, been conducted with the basic assumption that the range of the exponent for all bases be equivalent. This leads to comparisons then of formats where the number of bits set aside for fraction and exponent vary depending upon the base being used. We believe that this type of comparison, although theoretically pleasing and possibly applicable for future design, does not aid the computer buyer in determining which existing com-

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puter would be numerically best for his application. The majority of existing computer systems using 32 bit floating point formats, either allow 24 bits for the fraction, including sign bit, and 8 bits for the exponent, (24 X 8), or 25 bits for fraction and 7 for exponent (25 X 7), independent of the base being used. Moreover, hardware configurations, byte addressability and compatibility with existing systems will most probably dictate the continued usage of such formats. This paper is intended to give more insight on the attainable numerical accuracy of floating point systems with the word format remaining constant. Static properties of the (24 X 8) word format are compared with various choices of base. Finally, a table comparing various word formats and bases, along with examples of systems using such formats, are given.

*Preliminaries*

We will assume that we have a normalized sign-magnitude floating-point representation scheme with 32 bits/word. Unless specified otherwise each word contains a 24 bit fraction, f and an 8 bit exponent, e, with a base of $\beta$. Moreover, when using a base of 2 we will consider the case where the first bit is implicit, Brent (1973). Thus, if the implicit first bit technique is used, we will let the variable p = 2, otherwise p = 1. We will then consider systems of the following type $(\beta,p) = (2,1), (2,2), (4,1)$ and $(16,1)$, where $\beta$ is the base being used.

*Static Characteristics*

Cody (1973) has studied floating point systems with the range of exponents remaining the same, comparing (23 X 9), (24 X 8) and (25 X 7) systems with $(\beta,p) = (2,1), (4,1)$ and $(16,1)$ respectively. In this section, we wish to make the same type comparisons with a common (24 X 8) format for all bases. In our study of numerical errors, we will let r represent an equivalence class of real numbers and assume our error occurs in representing an element of r. This error will be referred to as the representation error. Assuming the logarithmic probability distribution for floating point numbers (Hamming, 1970), the average relative representation error ARRE, is given by

$$(1)\quad \mathrm{ARRE}(\beta) = \frac{\beta - 1}{4 \cdot 2^{t} \ln \beta}$$

where t = number of bits used for fraction, not including sign bit.

For the (24 X 8) word structure t = 23 if p = 1 and t = 24 if p = 2, that is if the implicit bit system is used. The maximum relative representation error (MRRE) is given by

$$(2)\quad \mathrm{MRRE}(\beta) = 2^{-t-1}\beta.$$

Table 1 gives the values of ARRE and MRRE for the (24 X 8) word format with $(\beta,p)$ equal to (2, 2), (2, 1), (4, 1) and (16, 1). As can be seen, and we might

add as expected, $(\beta, p) = (2, 2)$ gives the best results followed by the (2, 1), (4, 1), and (16, 1) system.

TABLE 1
ARRE and MRRE for Fixed Format

| Format | t | $\beta$ | p | ARRE | MRRE |
|---|---|---|---|---|---|
| (24 X 8) | 24 | 2 | 2 | $.180 \times 2^{-23}$ | $1 \times 2^{-24}$ |
| (24 X 8) | 23 | 2 | 1 | $.361 \times 2^{-23}$ | $2 \times 2^{-24}$ |
| (24 X 8) | 23 | 4 | 1 | $.541 \times 2^{-23}$ | $4 \times 2^{-24}$ |
| (24 X 8) | 23 | 16 | 1 | $1.353 \times 2^{-23}$ | $16 \times 2^{-24}$ |

As a point of comparison, Table 2 shows values for ARRE and MRRE where the range of the exponent remains constant.

Here $t = 23$ if $\beta = 2, p = 2$
$t = 22$ if $\beta = 2, p = 1$
$t = 23$ if $\beta = 4, p = 1$
$t = 24$ if $\beta = 16, p = 1$.

Notice that comparisons using Table 2 might lead to entirely different conclusions than if using Table 1. From Table 2 the MRRE of the (2, 1) and (4, 1) systems are the same and the ARRE of the (2, 1) system is greater than that for the (4, 1) and (16, 1) systems.

TABLE 2
ARRE and MRRE for Formats with Same Exponent Range

| Format | t | $\beta$ | p | ARRE | MRRE |
|---|---|---|---|---|---|
| (23 X 9) | 23 | 2 | 2 | $.361 \times 2^{-23}$ | $2 \times 2^{-24}$ |
| (23 X 9) | 22 | 2 | 1 | $.722 \times 2^{-23}$ | $4 \times 2^{-24}$ |
| (24 X 8) | 23 | 4 | 1 | $.541 \times 2^{-23}$ | $4 \times 2^{-24}$ |
| (25 X 7) | 24 | 16 | 1 | $.676 \times 2^{-23}$ | $8 \times 2^{-24}$ |

Conclusions using Table 2 might be that there is little difference between the (2, 1) and (16, 1) systems and a slight advantage in the ARRE of (4, 1) over (2, 1). This conclusion obviously could not be reached using Table 1.

Another quantity used in comparison of floating point systems is the variance of the relative error

$$\epsilon = E/f$$

where for our case E is the error in representation due to rounding the fraction

to t bits. E is assumed to be uniformly distributed in $[-2^{-t-1}, 2^{-t-1}]$. The equation for the variance is given by [2]

$$(3)\quad \sigma^2(\epsilon) = \frac{\beta^2 - 1}{10 \cdot 2^{2t} \ln\beta}$$

it should be noted that the mean of $\epsilon$ is 0. Also, if the particular system truncates rather than rounds, the mean is not zero and equation (3) changes. However, the comparisons of these variables give similar results.

Table 3 allows comparisons of $\sigma^2(\epsilon)$ for the various (24 X 8) systems, while Table 4 gives similar results maintaining equivalent sized exponents. As in the cases of comparing the ARRE and MRRE, two different conclusions could be obtained using the separate tables.

TABLE 3

Variance of Relative Error for Fixed Format

| Format | t | $\beta$ | p | $\sigma^2(\epsilon)$ |
|---|---|---|---|---|
| (24 X 8) | 24 | 2 | 2 | $.045 \times 2^{-46}$ |
| (24 X 8) | 23 | 2 | 1 | $.180 \times 2^{-46}$ |
| (24 X 8) | 23 | 4 | 1 | $.451 \times 2^{-46}$ |
| (24 X 8) | 23 | 16 | 1 | $3.832 \times 2^{-46}$ |

TABLE 4

Variance of Relative Error for Formats with Same Exponent Range

| Format | t | $\beta$ | p | $\sigma^2(\epsilon)$ |
|---|---|---|---|---|
| (23 X 9) | 23 | 2 | 2 | $.180 \times 2^{-46}$ |
| (23 X 9) | 22 | 2 | 1 | $.721 \times 2^{-46}$ |
| (24 X 8) | 23 | 4 | 1 | $.451 \times 2^{-46}$ |
| (25 X 7) | 24 | 16 | 1 | $.958 \times 2^{-46}$ |

*Comparison of Common Formats*

Because of the various choices of word formats and bases, comparison of numerical accuracy of all systems cannot be simply performed. Choosing as standard a common range for the exponent or common word format, leads to results which for the computer buyer, may be somewhat misleading. For existing systems, however, comparison of attainable accuracy can be simply accomplished since the format and base have been set.

Table 5 gives values for the ARRE, MRRE and $\sigma^2(\epsilon)$ for 3 of the most common floating point formats and bases. In addition, examples of computer sys-

TABLE 5

Comparisons of Characteristics of Various Existing Systems

| Format | Base | ARRE | MRRE | $\sigma^2(\epsilon)$ | Examples |
|---|---|---|---|---|---|
| (25 X 7) | 16 | $.676 \times 2^{-23}$ | $8 \times 2^{-24}$ | $.958 \times 2^{-46}$ | NOVA, UNIVAC 1616 |
| (24 X 8) | 2 | $.361 \times 2^{-23}$ | $2 \times 2^{-24}$ | $.180 \times 2^{-46}$ | Honeywell 316 |
| (24 X 8) | 2 (Implicit Bit) | $.180 \times 2^{-23}$ | $1 \times 2^{-24}$ | $0.45 \times 2^{-46}$ | PDP-11 |

tems using such floating point structures are listed. These examples are certainly not exhaustive.

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FIRST SOUTH TEXAS RECORDS OF *PAPPOGEOMYS CASTANOPS*– Although the chestnut-faced pocket gopher has been reported in west Texas (Davis, 1974, Tex. Parks and Wildlife Dept. Bull. 41:170; and Thornton and Creel, 1975, *Southwestern Naturalist*, 20:272), no previous records have been noted from south Texas. Blair (1952, *Tex. J. Sci.* 4:230) failed to record this species. Three specimens of *Pappogeomys castanops* were collected during a mammal survey of the National Audubon Society Sabal Palm Grove Sanctuary at Southmost, Texas. These animals are in the collection of the Museum of Zoology at Texas Wesleyan College.

Two adult females (TWCMZ-1449 and TWCMZ-1674) and 1 juvenile female (TWCMZ-1833) were collected 6 mi (9.7 km) southeast of Brownsville, Cameron County, Texas. The first adult catalogued was taken on 11 October 1974. The second adult and the juvenile were collected on 8 January 1976.

The gophers were all taken in mixed short grass with deep sandy soil north of the Sabal Palm forest. Numerous burrows were observed although none were noted during a similar visit to the site in 1972. The nearest previously reported site was south of the Rio Grande River in Tamaulipas, Mexico (Russell, 1968, *Univ. Kans. Publ. Mus. Natur. Hist.*, 16:580). Associated mammals taken from the site include *Rattus rattus, Mus musculus, Reithrodontomys fulvescens, Cryptotis parva, Liomys irroratus, Peromyscus leucopus, Perognathus hispidus,* and *Sigmodon hispidus.*–*Arthur G. Cleveland, Department of Biology, Texas Wesleyan College, Fort Worth 76105.*

THE LEVEL OF UNDERSTANDING OF ALGEBRA AND TRIGONOMETRY BY STUDENTS OF FRESHMAN PHYSICS– The high school graduate is required to use his education in a variety of ways, and in many cases can minipulate circumstances to avoid having to expose academic deficiencies. However, those students who attend college in one of the preprofessional courses of study must encounter a survey course in physics in a head on confrontation. This is generally considered to be a backbreaking experience, and dropout and failure rates in these courses higher than 50% are accepted by college faculty without concern or response.

A few years ago we became concerned that factors other than the topics covered in the course were contributing substantially to the difficulty, and began to seek those parameters which might fall into this category. As a result, a 26 question multiple choice test of 13 identifiable algebraic and trigonometric skills was developed. This paper reports the result of that test when given to 843 students of freshman physics in the fall of 1976.

## DISCUSSION

The topics tested are given in Table 1. Each topic had 2 questions, and those students who correctly answer both questions on a given topic are said to "Understand" that topic. Those who miss both questions are classified as "Do not understand", and those who have only one correct are called "Not sure".

Table 1 also lists the % response of the students who finished in the top 20 percentile of one section of 233 students. The other sections did not use computer grading so the correlation of final standing with the mathematics test is not readily available. We feel that the sample of 233 students is large enough to have meaning.

The responses should be evaluated in terms of the test being multiple choice, 5 answer choices/question. Guesses would fall on top of correct answers and skew figures up. To counter that, the test was given on the first class day without prior warning and many students may have done better if some review had preceded the examination.

TABLE 1

| | Group As A Whole | | | Students Who Finished In Top 20 Percentile | | |
|---|---|---|---|---|---|---|
| | UNDERSTAND | NOT SURE | DO NOT UNDERSTAND | UNDERSTAND | NOT SURE | DO NOT UNDERSTAND |
| 1. Subtraction of negative numbers | 72% | 15% | 13% | 77% | 16% | 7% |
| 2. Linear algebraic equation in one unknown | 69% | 19% | 12% | 86% | 7% | 7% |
| 3. Square roots of products and sums | 80% | 6% | 14% | 93% | 6% | 1% |
| 4. Quadratic formula | 56% | 23% | 21% | 74% | 12% | 14% |
| 5. Linear parametric equation in two unknowns | 39% | 8% | 53% | 51% | 4% | 45% |
| 6. Parametric equations in two unknowns, one a quadratic | 22% | 12% | 66% | 39% | 16% | 45% |
| 7. Graphs and tables | 84% | 6% | 10% | 88% | 6% | 6% |
| 8. Definition and use of sine | 30% | 32% | 38% | 53% | 29% | 18% |
| 9. Definition and use of cosine | 38% | 24% | 38% | 44% | 19% | 37% |
| 10. Definition and use of tangent | 31% | 26% | 43% | 47% | 16% | 37% |
| 11. Trigonometric functions of angles greater than 90° | 24% | 13% | 63% | 25% | 21% | 54% |
| 12. Pythagorean theorem | 53% | 26% | 21% | 63% | 30% | 7% |
| 13. Radian measure | 60% | 15% | 25% | 57% | 18% | 25% |

We also surveyed the mathematics background on the students in the section of 233. Of that number, every student had had at least 1 semester of high school algebra and only 9 had less than 1 year. Fifty did not take high school trigonometry, but only 5 had not completed trigonometry in college before taking this test. We feel that the ability to use the mathematics expected to be a part of an algebra and trigonometry course was inadequate, especially for topics 6, 8, 9, 10 and 11. Topic 11 stands out because even better students did not fare well.

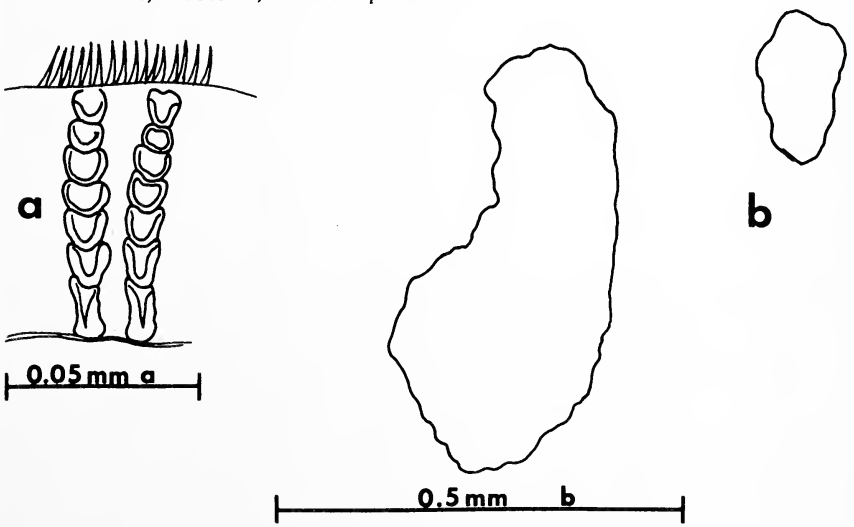
## CONCLUSION

The University of Houston is a large, urban, commuter campus. The mean SAT of entering freshman was 922 in 1975 (the last year for which figures are available). Many of these students come from some of the most reputable school districts in the state of Texas. Therefore the sampling is considered to be typical. It is obvious that, for this group, the lack of ready skills in algebra and trigonometry is contributing to their difficulties in the study of physics.

The authors will be happy to send a copy of the test on request.–*H.T. Hudson, and R.M. Rottmann, Physics Department, University of Houston, 77004.*

THE OCCURRENCE OF *ARGULUS BICOLOR* BERE, 1936 ON *TRACHINOTUS CAROLINUS* (LINNAEUS) FROM THE TEXAS COAST—During July 1975 1 male *Argulus bicolor* (Bere, 1936) was found on the first gill arch of a Florida pompano, *Trachinotus carolinus* (Linnaeus). The pompano came from a sample taken in the surf off South Padre Island, Texas.

This is the first occurrence of *A. bicolor* from the Texas coast, the previous range being from North Carolina to Louisiana (Cressey, 1972, *Water Pollution Control Research Series,* 18050 ELD-2171:14 pp.) *T. carolinus* is a new host for this species. Cressey (1972) reported the following genera as hosts: *Strongylura, Morone, Gobionellus, Micropogon, Scomboromous, Dorosoma,* and *Rhinoptera.*



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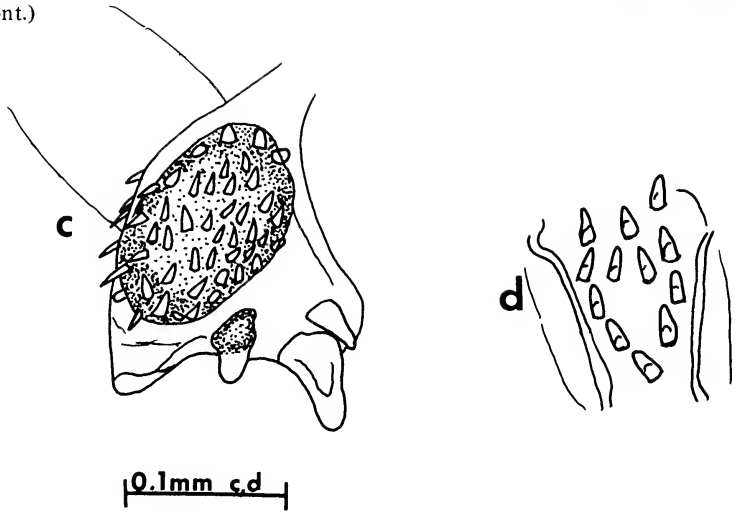


Figure 1. *Argulus bicolor*, a) Supporting rods sucking discs; b) Respiratory areas – vential view; c) Basal plate of maxilla; d) Base of mouth tube with spines.

The principal character used to identify the animal was the respiratory areas (Fig. 1b). The supporting rods of the sucker wall (Fig. 1a), the teeth on the basal plate of the maxilla (Fig. 1c) and the spines on the base of the mouth tube (Fig. 1d) were instrumental in the final identification. The keys used for identification were Cressey (1972) and Wilson (1940, *Proc. U.S. Natl. Mus.*, 88(3087):459.–*Timothy L. Jones, Southwest Research Institute, 3600 Yoakum Blvd., Houston 77006, and Ronald C. Circe, Box 6010, No. 339, Texas A&I, Corpus Christi 78411.*

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## COVER PHOTO

Location map. The study area is centered in the Waco urban region and encompasses McLennan County, Texas and its immediate vicinity.

by R.G. Font, pp. 187-194

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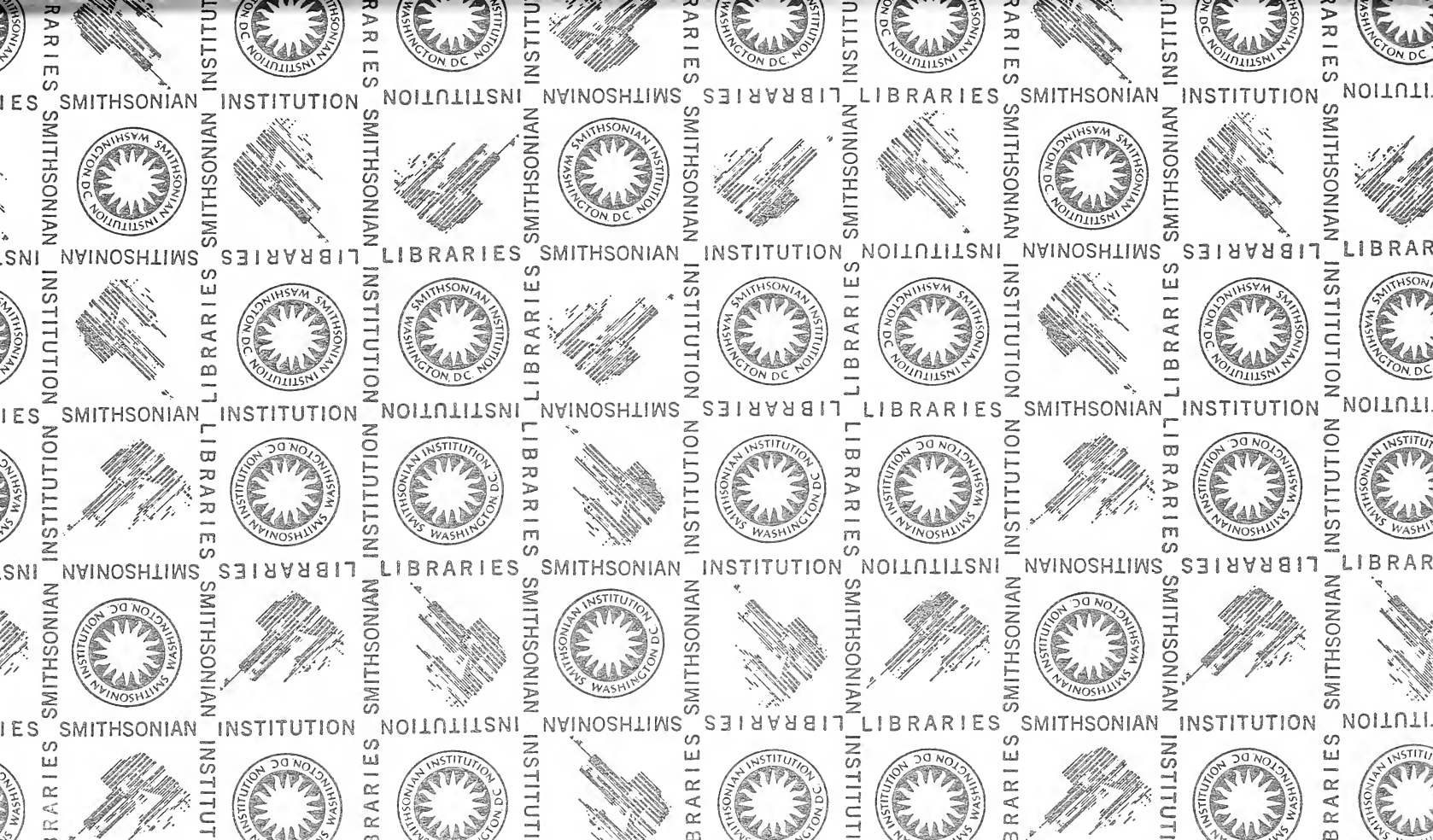
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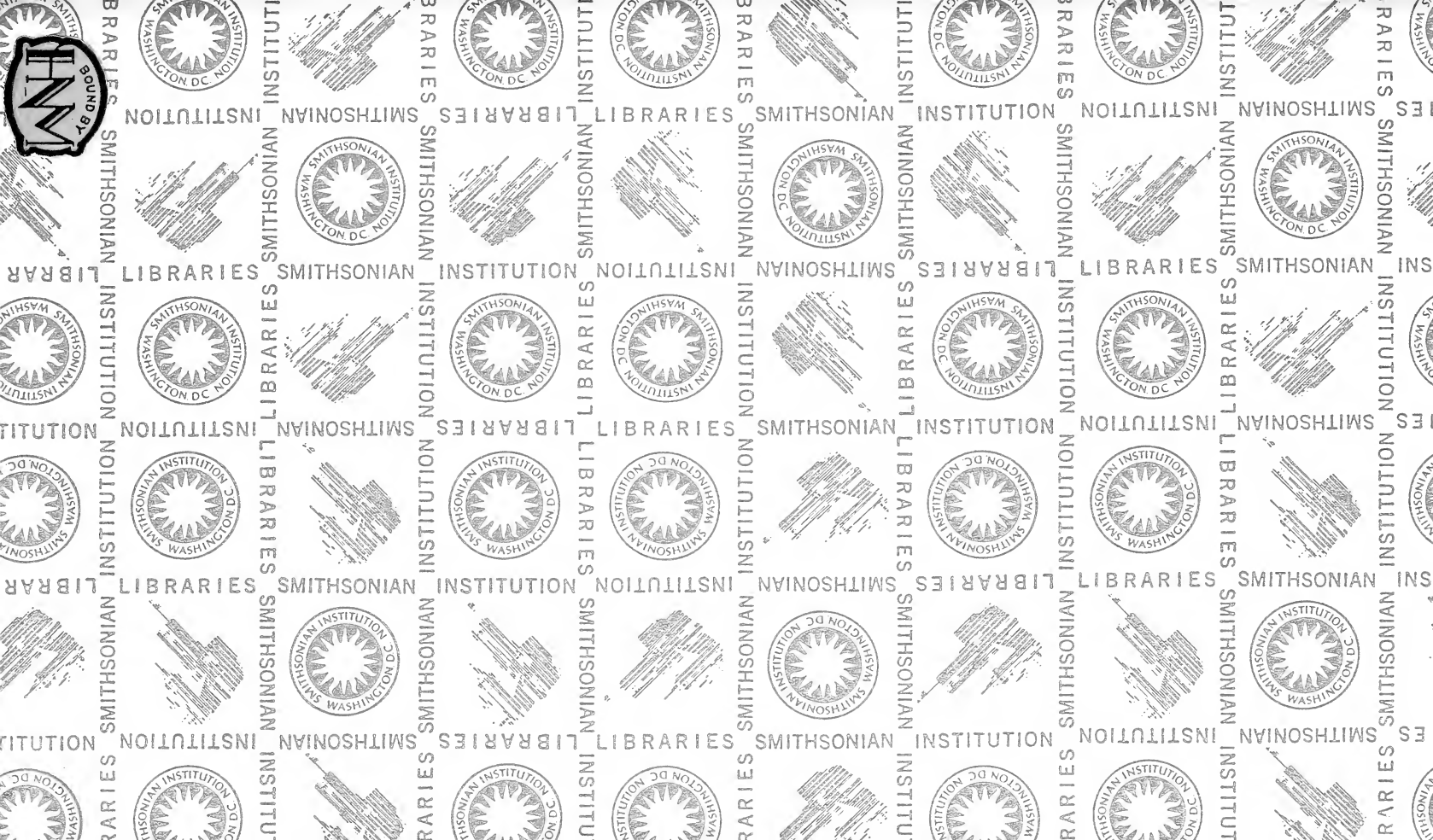
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